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Stress, enrichment and the welfare of domestic cats in rescue shelters

Stress, enrichment and the welfare of domestic cats in rescue shelters

By

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Abstract

Domestic cats relinquished to shelters are thought to experience a high level of acute stress upon admission, and then undergo a generally lower level of chronic stress for weeks or months afterwards. This thesis contains three studies aimed at refining measures of cat welfare, and estimating the impact of physical and social enrichment on those measures. The first study described the time-course of stress measures in cats following admission to a rescue shelter, and their subsequent move from one part of the shelter to another. Both urinary Cortisol:Creatinine (CC) and the behavioural Cat-Stress-Score (CSS) fell over time; they were positively correlated within cats, though negatively correlated between-cats, suggesting differing coping strategies. The second study tested the efficacy of open-sided boxes 26x36x26cm, and of increased social contact with a human on relieving stress. CC again fell over time, but no effect of box or social contact on CC was found. Boxes were used by the majority of cats, and reduced CSS, with the greatest effect occurring on the day of admission and continuing until at least day 7. Increased social contact with a known human also reduced CSS, but only when measured by the human who gave the contact, and not when measured remotely. It had no effect on approach tests by familiar or unfamiliar humans. The third study found that adding boxes of a slightly different design to the pens of long-stay cats did not significantly reduce their CSS, though the boxes were used extensively. However, removal of the boxes after two days availability caused an increase in CSS, suggesting that boxes are a valuable resource. Boxes made long-stay cats less likely to make either an approach or withdrawal during approach tests.

Dedication

"We dance around in a ring and suppose,
But the secret sits in the middle and *knows*."

Robert Frost

For Hilary, my mother and Guy, my father

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Author's declaration

I declare that the work in this dissertation was carried out in accordance with the regulations of the University of Bristol. The work is original, except where indicated by special reference in the test, and no part of the dissertation has been submitted for any other academic award. Any views expressed in the dissertation are those of the author.

SIGNED:.. DATE: 23/12/05.........

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Chapter 1 - Introduction

1.1 Introduction

There is much anecdotal and empirical evidence that admission to a shelter is stressful to domestic cats (*Felis sylvestris catus)*. For example, most cats in boarding catteries show signs of stress for at least the first four days after admission (Kessler and Turner 1997). These authors reported that some cats remained very tense for at least the first fortnight after admission. Similar findings were shown by Smith *et al.* (1994) for cats in a rescue shelter. Rochlitz *et al.* (1998a) found that cats admitted to quarantine catteries took five weeks to show evidence of adaptation to their new environment.

This experience occurs to many of the 8 million domestic cats in Great Britain (PFMA 1999), of which approximately 1 million are feral or 'unwanted' cats (Rees 1982). Although the exact number of cats entering animal shelters in Britain is unknown, figures from Cats Protection and the RSPCA (the two largest UK charities that shelter cats) and other shelters were collected by Rochlitz (2000a). These suggest that around 150,000 cats may be relinquished to cat shelters each year. Since well-cared for domestic cats can expect to live for 12 years (McCune 1999) and many owned cats are put into boarding catteries for a few weeks, some 10% of cats may have experience of cat shelters or similar accommodation.

This introduction will cover the history and basic behaviour of the domestic cat, followed by an overview of the methods used in the study of animal welfare. The current research on cat welfare in shelters will then be discussed, followed by a review of the experimental techniques suitable for cat welfare research.

1.2 Domestic cat origins and behaviour

The ancestor of the domestic cat is believed to be the wild cat *Felis sylvestris* (Clutton-Brock 1987). *F. sylvestris* is a single species that varies continuously through its range (from Scotland to Georgia, Africa, SW Asia and N India). There are 3 subspecies - the Scottish or European wild cat *(F. s. sylvestris)* the African wild cat (*F. s. lybica*) and the Indian desert cat *(F. s. ornata)* (Kitchener 1991). The *lybica* form is thought to be the most adaptable to living alongside humans, and is likely to have been the ancestor of the domestic cat (*F. s. catus*). This is supported by alloenzyme studies (Randi and Ragni 1991). The process of domestication probably started in Egypt around 4000 BC (Clutton-Brock 1987) though cats still retain many of their wild behaviour patterns (Bradshaw 1992). Their distribution is now global, with the highest numbers of cats per human occurring in Western Europe, the USA and Australasia (Anon. 1990).

Studies of feral domestic cats show that their spacing is largely determined by the environment. Under some conditions such as certain desert islands (van Aarde 1978, Corbett 1978) they may be strictly solitary for most of the year, or they may live in colonies centred around shelters or concentrations of food (frequently left by humans, whether purposely for cats or not) in farms, dockyards and city parks (Kerby, 1987, Izawa *et al.* 1982, Natoli 1985a). Colony densities range from 1 to 2000 cats per km^2 (Izawa *et al.* 1982, Kerby and Macdonald 1988).

Whether colonies or exclusive home ranges occur depends on the clumping of food resources, with clumping favouring colonies (see Liberg *et al.* 2000). Free-living cats tend to single out particular partners for interaction, which are often those that they choose to rest beside or in contact with. Interactions are clearly structured (e.g. Bradshaw and Brown 1992), and do not support the idea that colonies are simple aggregations around food sources. These colonies may contain both males and females, with related females and their young forming matrilines - cats more closely related tend to be most affiliated to each other (Kerby 1987). Some 'peripheral' males may be loosely attached to the group, and form large territories which may overlap those of other males and contain many colonies. A study of feral colonies (Kerby

1987) suggested that higher ranking female cats and their matrilines monopolised food and the best sites for raising kittens to a sufficient extent to increase high ranking females' reproductive success.

The cat's perceptual world is quite different from that of humans. At low frequencies the cat's auditory thresholds (lowest amplitude detected) are broadly similar to our own, but while our thresholds decrease above 4 kHz, a cat's only start to decrease at around 10 kHz, and cats can still usefully detect sounds up to 60 kHz (see Bradshaw 1992). Cats' vision functions at lower light intensities than ours, and is highly tuned to detect rapid movements. Evidence suggests that cats cannot focus on objects closer than 25cm (Elul and Marchiafava 1964), and although they can see colours, they have only green and blue sensitive receptors so cannot see red and perceive red objects as darker than humans do (Loop *et al.* 1987). Perceptual dominance favours such cues as brightness and pattern over colour. Their sense of smell is far more sensitive than that of humans, and they have a vomeronasal organ which is used to analyse the excreta and secretions of cats and other animals (Clutton-Brock 1993).

Cats can communicate in many different ways – a large part of the cat's repertoire consists of vocal signals, which can convey a desire for contact (e.g. purr) or be a greeting, sexual, aggressive or defensive signal (e.g. meow, male 'mowl', growl or hiss respectively) (Bradshaw, 1992). The form of many of these calls may be shaped or trained by the cat – owner relationship.

Visual signals are also used in various contexts. For example, the vertical tail-up position can signify a greeting, play or, in females, a sexual approach; and a lowered posture in agonistic encounters indicates submission / fear (Leyhausen 1979, Bradshaw 1992).

Olfactory communication is known to be important to cats, though its function is not clearly understood, being possibly related to territory marking or sexual behaviour (Natoli 1983b). Urine marking is common in both wild living cats and domestic cats of both sexes, and cats have a variety of scent glands which are used to deposit marks on prominent objects at around head-height.

The territorial behaviour of owned domestic cats has not been studied in detail, though competition between house cats for foraging space is frequently observed when a new cat is introduced to an area with existing cat territories. Such territories may not be continuous and cats may take long and circuitous routes, or hurry past, territories owned by other cats (Brown and Bradshaw, unpublished data in Bradshaw 1993). Females and castrated males have similar sized home ranges (female median 0.053ha, castrated male 0.076ha, Chipman 1990), though entire males have far larger territories (median .88ha).

1.2.1 The human - cat relationship

Kittens have a 'sensitive phase' at between 2-7 weeks old when they will socialize towards any object with the right stimulus qualities (these include factors such as size, movement, form and texture) they come in contact with (Karsh and Turner 1988). Humans fulfil these stimulus qualities, and kittens will readily socialise to them during this time. The degree of socialisation depends on the amount of time spent with the kitten – Karsh and Turner found that a kitten handled for one hour a day will go directly to a familiar person, climb into their lap and either play or go to sleep. Kittens handled for 15 minutes a day tend to approach, head rub, then move off. There is an effect of the number of handlers also – cats with only one handler can be held twice as long on average by that person than by other humans. Cats with four handlers will stay with any person, including a stranger, for the same time as a 'one-person' cat will stay with its handler. Some element of generalisation appears to occur after a few humans have been met. Socialised cats are also quicker to approach and touch a test person than non-socialised ones (McCune 1995). This handling affects only the cat's perception of humans, and does not include a more general boldness effect such as curiosity towards novel objects (McCune 1995).

Owned domestic cats appear to perceive their human owners as part of their social group (Bradshaw 1993), and cats which live with human families will, when hungry, direct interactions towards the human that feeds it (Bradshaw and Cook 1996), though they are likely to be just as affectionate towards other family members at other times. The behaviour patterns cats use towards humans are broadly similar in form to those used in cat-cat social behaviour (Bradshaw 1992).Some human directed patterns (such as miaowing) have been classified as infantile and may continue into adulthood because humans respond to them. Allorubbing is commonly directed by cats towards owners and has been classed as amicable and mildly subordinate.

Mertens and Turner (1988) looked at the initial interactions during the first 10 minutes after contact between cats and humans who were unknown to them. In the initial 5 minutes, when the humans were instructed not to interact with the cat, cats vocalised more frequently than in the second 5 minute period, in which full interaction was allowed. These vocalisations were interpreted as the cats' attempts to induce contact. The second phase had a higher rate of head rubbing than the first. Differences in behaviour shown to adults and children, and to either sex, were not apparent during the first phase but appeared once the humans were allowed to start interacting – the cats then reacted to differences between the groups and individuals. For example, cats were more likely to come close to a human if he/she initiated contact.

Turner (1991) analysed behaviours between 158 female owners and their cats in a home setting, as recorded by the owners over a three day period. Looking at the relative proportions of interactions attempted by owner and cat, the higher the proportion that were initiated by the owner, the shorter the overall (daily) interaction time with the cat. The higher the proportion that were initiated by the cat, the longer the interaction time with the cat. This suggests that if the cat is left to initiate contact when it wishes it, the longer the overall contact will be than if the human asks it to interact when it does not with to. However, this result could also be due to particularly friendly cats making more attempts, and less friendly cats making less initiations. Complying with the other partner's wishes (i.e. interacting when the other partner initiates) is positively correlated for each female-cat pair, so if the woman complies with the cat's wishes to interact, the cat complies with the woman's wishes at other times.

The link between humans and their cats can be very strong. Bahlig-Pieren and Turner (1995) report that numerous surveys indicate that over 90% of cat owners believe they can sense the mood of their pets, and *vice versa*. Bonas *et al.* (1998a) concluded that the cats provide owners with emotional support, particularly in buffering against deleterious events. This differs from human support, with human-human relationships providing more companionship (Bonas *et al.* 1998b). Similarly, Stammbach and Turner (1999) conducted a questionnaire study on female, human cat owners and found that whilst cats can substitute for a human in the owner's social support network, in most cases they provide a source of emotional support in addition to that from humans.

Serpell (1996) found that most (78%) pet owners class themselves as very attached rather than moderately attached to their pets (both cats and dogs, though no significant difference was found between cats and dogs, and no owners reported less than moderate attachment). There was no difference between the two classes in satisfaction with the pet. The main discrepancies between an owner's 'ideal' cat and their actual cat were: nervousness / fearfulness, excitability, lack of obedience, lack of playfulness, lack of affection, lack of intelligence and aggression towards known humans. Although the 'ideal' conceptions of cat behaviour bore no relationship to the level of attachment, the discrepancies between 'ideal' and actual did, with large discrepancies correlating with moderate attachment. Some owners may have unrealistic expectations of cats (Patronek *et al.* 1996), and it is such factors that may contribute to a cat's relinquishment to a cat shelter.

Turner and Staambach-Geering (1990) asked 150 owners to rate their cats in terms of character traits (both positive and negative). They found that owners' affection for their cat was most strongly correlated with the cat's perceived affection for the owner. Both of these traits were positively associated with high ratings for cleanliness and predictability. Unwelcome behaviours such as urine spraying and restlessness during the night were negatively correlated with the owner's level of affection towards the cat.

1.2.2 When the relationship breaks down - reasons for relinquishment to rescue shelters

People relinquish cats to shelters for a number of reasons. In the United States, Luke (1996) listed the reasons given by owners for cats being admitted to shelters run by the Massachusetts Society for the Prevention of Cruelty to Animals in the years 1992-4: unwanted / stray kittens were 42%, adult strays 17%, moving house / landlord problems 10%, behaviour problems 8%, not interested in owning cat anymore 7%, financial difficulties 6%, allergies 4%, owner requested euthanasia of cat (reason for request not given) 7%. Casey and Vandenbussche (2003) collected data on relinquishment to 14 Cats Protection branches in the UK, and found that the greatest numbers of admissions were as strays or transfers from other branches / charities (39%). Of those that were relinquished by owners, moving house / landlord problems were 17%, behavioural problems 14% (5% cat-cat aggression, 3% aggression to humans, 2.5% nervous / fearful, 2% urinating or defaecating indoors), allergies 12%, death of owner 6%, pregnancy / young children 4%, financial difficulties 3%, domestic problems 3%.

Of the above, moving house, behaviour problems, lack of interest and financial difficulties will rarely be absolute reasons – the owner could choose not to move house or put up with / treat the behaviour problem, so the level of attachment with the cat might affect the likelihood of these resulting in relinquishment of the cat.

Other studies tend to concentrate on perceived problems of owners without looking directly at admissions to shelters. While not quite as relevant, these may be more truthful (owners may feel the need to invent a 'good reason' for giving an unwanted cat to a shelter). Cases referred to the UK Association of Pet Behaviour Counsellors (APBC) in 2004 included aggression towards other cats (19%), indoor marking and house training problems (42%), aggression towards people 12%, and fearful behaviour (7%). Casey (2001) found that anthropogenic reasons (things which affect the owners directly, such as such as inappropriate elimination or marking) seemed to dominate referral cases, with fearful or avoidance related behaviours being underrepresented when compared to the occurrence in the population of owned cats in general. Vandenbussche *et al.* (2003) found that owners who reported more frequent behavioural problems mentioned a lower degree of comfort received from their cat.

These results suggest that cats given to shelters may be more likely to be strays or have behavioural problems than their proportions in the general population would suggest.

1.2.3 Forming relationships – factors affecting adoption of cats from shelters

The aim of most cat shelters is successful rehoming of the cats in their care, which is dependent on visiting humans wanting to adopt them. Many shelters known to the author generally have few free spaces and have waiting lists of owners who want to relinquish their pets. This makes rehoming cats to free up spaces in the shelter of paramount importance. Vandenbussche (2001) and Vandenbussche *et al.* (2002) studied 89 cats in a Cats Protection shelter and found that the age, colour and Cat Stress Score (which rates the cat's stress according to postural and behavioural elements, see section 1.4.1) were related to their chance of being rehomed – adult cats (over 2 years old) were less likely to be rehomed than younger cats, tabbies most likely to be reserved and black cats least, and cats with higher Stress Scores less likely to be reserved. Sex of the cat, its position in the cage and response to an approach test were not significantly related to reservations. Although cats which played were more likely to be reserved, this measure may be confounded with age.

Interviews with potential owners (N=92) visiting the shelter showed that age (62%), physical appearance (29%), character (25%) and sex (18%) were most frequently reported as the most important feature of a cat. When asked about character, 'sociable to people' (58%) and 'sociable to other pets' (23%) were expressed most often.
Of the 57 people in the sample who reserved a cat, 73% of them emphasized the personality of the cat as the reason for choosing it. Physical appearance was also included in decisions for 46% (more than one reason could be given), and only 19% mentioned age as a reason despite the reservation data suggesting that age is the most important factor.

Follow-up questionnaires were sent to people who had selected a cat. Looking at whether the cat reserved matched the owner's preferred features, 60% chose a cat from the age range they wanted, while only 16% chose a cat of the colour they preferred. Only 46% of responders mentioned the same character traits in their chosen cat as the ones they had expressed a preference for. Character was frequently mentioned in the reasons why people chose that particular cat which shows the importance of character despite the approach tests' lack of significance.
However, no correlation was found between the degree of matching (expressed preference compared to actual cat) and the level of emotional support owners received from their cat.

Cats are sometimes returned to the shelter due to the adoption not working out - aggression towards other cats was the greatest behavioural cause of cats returning to shelters after homing (Casey and Vandenbussche 2003).

1.3 Animal Welfare

Broom (1986) defined the welfare of an individual as 'its state as regards its attempts to cope with its environment'. Animals have an array of responses to cope with adverse conditions, such as running away from a predator or searching for water when thirsty. If these responses do not work (so that the animal continues to be chased or thirsty) it is failing to cope and its coping systems will be overtaxed. When challenged beyond its capacity to cope, an animal is said to be undergoing stress (Terlouw *et al.* 1997). It should be noted that 'natural selection maximises fitness, but not necessarily the well-being of organisms' (Hofer and East 1998).

Although some authors (such as Broom) consider that animals can have poor welfare without suffering (e.g. from a tumour they cannot feel), most concerns over welfare arise from the possibility that animals suffer as a result. One of the main reasons for accepting that animals can suffer is the 'argument by analogy' (Dawkins 1990, Sandøe and Simonsen 1992, Stafleu *et al.* 1992, Sherwin 2001). An analogy is drawn between situations causing, and the behavioural and physiological indicators of, suffering in humans, and similar situations and indicators in animals. This leads to the conclusion that the subjective experience of animals which are in such situations and which simultaneously present such indicators, may be analogous to our own (though see Sherwin, 2001). It should be clarified that suffering only occurs when noxious stimuli are prolonged - a mild itch does not cause suffering; severe itching which prevents an animal from sleeping or resting does (Dawkins 1990).

It is also likely that all vertebrate animals experience a state akin to anxiety, as they all possess specific receptors for anxiolytic benzodiazepine drugs (Rowan 1988). Whether the argument by analogy is accepted or not, the negative moral consequences of erroneously rejecting the argument by analogy are far greater than those of mistakenly accepting it (Stafleu *et al.* 1992).

Some ways of measuring welfare in general terms will now be discussed. Welfare measures for cats in particular will be dealt with in section 1.4.

1.3.1 Immunological measures, injury and disease

Some of the most obvious indications of poor welfare are disease and injury. The extent of damage and the degree of disturbance of physiological and behavioural processes give information about how poor welfare is (Broom 1991), and information about the severity of pain or suffering can also be obtained from these measures (Morton and Griffiths 1985). Immunosuppression may occur when animals are exposed to stress - for example, lambs (*Ovis aries*) show reduced lymphocyte responses to antigen challenges after restraint (Coppinger *et al.* 1991). Despite much research, the exact mechanisms underlying the immunosuppressive effects of stress are not known (see Terlouw *et al.* 1997 for discussion).

1.3.2 Physiological measures

The common physiological response to a variety of environmental stressors includes changes in plasma concentrations of certain hormones, and their effects on processes such as heart rate and glucose uptake by muscles. This is often termed the 'stress response', and in addition to physiological signs, includes behavioural responses such as running away (e.g. from a predator). The stress response is relatively nonspecific - many different stressors elicit a similar response (Selye 1950). Two endocrine systems, the sympatho-adrenal system and the hypothalamo-pituitary-adrenal axis (HPA axis), form the major components of the stress response (Stratakis and Chrousos 1995), and I shall deal with each of them in turn.

The sympatho-adrenal system

Within seconds of the brain perceiving a stressor, the sympathetic nervous system begins to secrete noradrenaline (termed norepinephrine in the USA), and the adrenal medullae located above the kidneys begin to secrete adrenaline (epinephrine). These hormones (termed catecholamines) cause the 'fight or flight' response (Cannon 1929) - increased heart rate, blood pressure and respiration rate, increased blood flow to the muscles from the trunk and increased blood glucose levels through the stimulation of glucagon release and the inhibition of that of insulin. All these changes except the increased blood flow to the muscles are relatively easy to measure. Since individuals will vary in these measures, it is desirable to obtain baseline values for each individual to compare with post stressor values.

The hypothalamo-pituitary-adrenal axis

Within minutes of the detection of a stressor, the adrenal cortex begins to secrete glucocorticoids (Figure 1.1, Nelson 2000). Corticotrophin-releasing hormone (CRH) is released from the hypothalamus into the portal blood system, which transports it into the anterior pituitary gland, where it stimulates the secretion of adrenocorticotrophic hormone (ACTH) into the general blood system. This stimulates the adrenal cortex to secrete glucocorticoids. These vary between species, with most rodents and birds secreting predominantly corticosterone, and primates and carnivores predominantly secreting cortisol (Stratakis and Chrousos 1995). Bush (1991) reports that corticosterone occurs in cats at concentrations of about 25% that of cortisol. These glucocorticoids affect metabolic pathways to ensure that the increased need for metabolic fuels is met. The levels of glucocorticoids can be measured and used as signs of stress. For example, Rushen *et al.* (2001) examined the effects of novelty stress on cows (*Bos taurus*). They found that cows in an unfamiliar room during milking had higher heart rates (due to activation of the sympatho-adrenal system) and higher plasma cortisol concentrations (due to activation of the HPA axis), than cows milked in a familiar room.

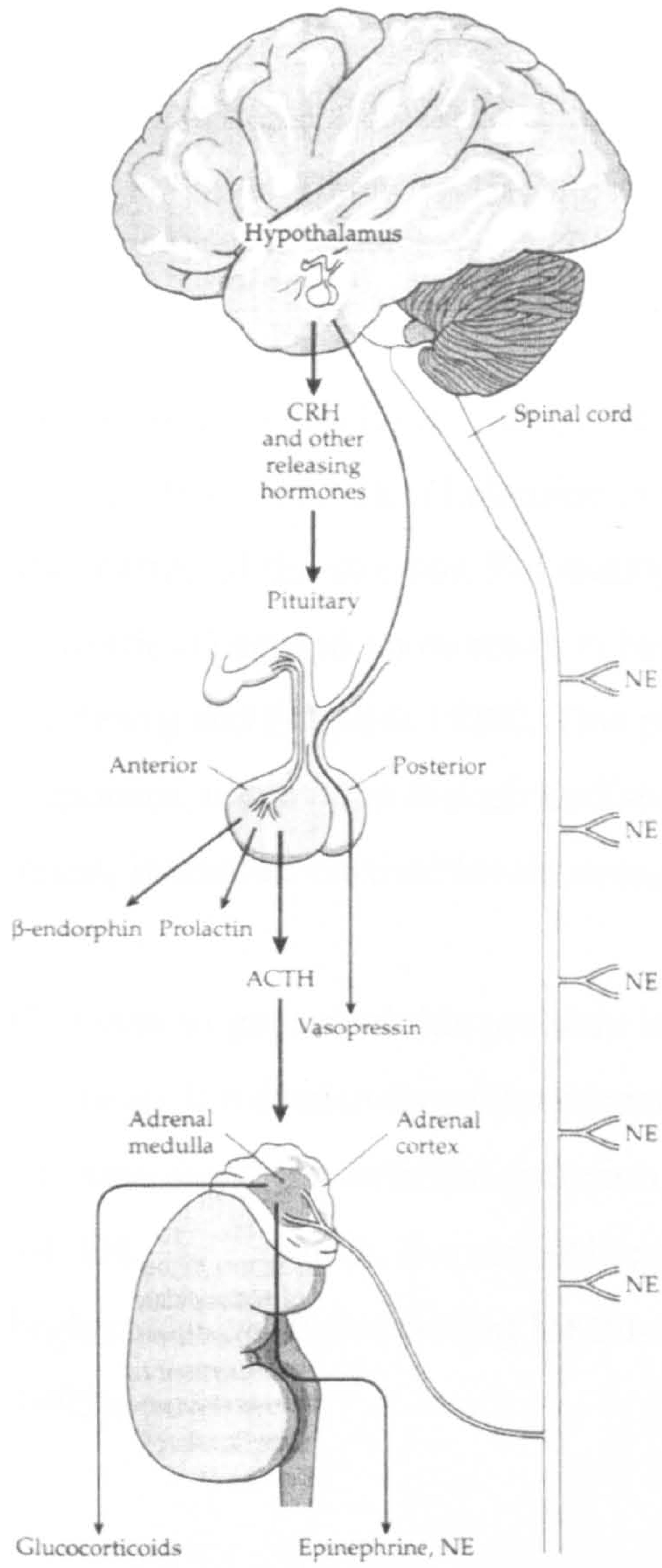


Figure 1.1 (Nelson 2000)
See text for details.
CRH = Corticotrophin-releasing hormone
ACTH = Adrenocorticotrophic releasing hormone
NE = norepinephrine

Chronic stress

The above are responses to acute stressors. Once the individual has escaped or nullified the stressor, the stress response will cease and the levels of catecholamines and glucocorticoids will return to normal. If the individual cannot escape the stressor, the stress response will continue. Prolonged activation of the stress response can lead to stress pathology, characterised by muscle wasting (due to prolonged glucocorticoid secretion), peptic ulcers, high blood pressure, and impaired disease resistance (Sapolsky 1992). However, absolute levels of physiological parameters have limited significance (Wiepkema and Koolhaas 1993), partly due to individual differences and possibly because the regulatory mechanisms that control neuroendocrine systems are highly dynamic and have a high degree of complementary plasticity. For example, a strong elevation of corticosterone may lead to a permanent decrease of corticosterone receptors in the hippocampus.

Due to these physiological adaptations, chronic stress causes overall plasma glucocorticoid and catecholamine concentrations to rise only briefly after the initial application of the stressor. For example, initial tethering of female pigs (*Sus scrofus domesticus*) caused an increase in basal plasma cortisol that lasted only a few days (Ladewig and Schmidt 1989). This plasticity, together with stressor-specific responses, means that though undisturbed levels of cortisol do not exclude chronic stress, increased cortisol levels strongly indicate it.

One way to get round this problem is to use an 'ACTH challenge'. Chronic stress often increases the sensitivity of the adrenal cortex to ACTH (Terlouw *et al.* 1997), so will increase the glucocorticoid secretion caused by experimental administration of ACTH. For example, the cortisol response to ACTH administration was significantly higher in pigs after tethering for three or four months than before (Janssens *et al.* 1995).

Problems with physiological methods

One problem with all measures of acute stressors is that the 'stress response' may not be as general a welfare measure as once thought. For example, a hen willow grouse (*Lagopus lagopus*) sitting on a nest will show *lowered* heart rate when approached by a predator, presumably to reduce the likelihood that a movement will make it visible to the predator (Gabrielsen *et al.* 1977). Further, the stress response is largely a preparation for activity, and occurs whether or not the activity is unpleasant. A rise in glucocorticoid levels (as well as increased heart rate) occurs in pleasurable situations such as sexual behaviour (Colborn *et al.* 1991). Behavioural measures can often be used to determine whether the stimulus is pleasant or noxious.

Furthermore, CRH, ACTH, and glucocorticoids are secreted in an episodic fashion throughout the day (Terlouw *et al.* 1997). With corticosterones being released seconds after stress, and glucocorticoids minutes after, samples should be taken as soon after the stressor as possible, and by as stress free a method as possible, such as a catheter. Problems such as these will be discussed more fully in section 5.

1.3.3 Behavioural measures

Many behavioural measures have been used as welfare indicators, though they are more specific to the species and situation under consideration than physiological ones. For example, we would not expect an animal to respond in the same way to dehydration as to an approaching predator (Dawkins 1998). Examples of behavioural measures include: in pigs, the speed of going from kneeling to lowering their hindquarters, and rate of slipping on different floor surfaces (Boyle *et al.* 2000); in rats (*Rattus norvegicus*), ambulation, rearing and defecation when subject to barium sulphate gavage (Alban *et al.* 2001); and the Rushen *et al.* (2001) study above, where cows defecated/urinated more, vocalized more and made more steps during milking in the unfamiliar room, all interpreted as signs of acute stress.

The absence of a behaviour can also be used: animals prevented from expressing a highly motivated behaviour are assumed to have poor welfare (Dawkins 1988). However, simply observing non-occurrence of a behaviour is insufficient, since the appropriate causal factors may be absent (Wood-Gush 1973). Experiments concerned with motivation can be used to assess this. The presence of vacuum activities, such as hens in wire floored cages going through the motions of dustbathing even though there is no loose substrate on the floor and no dust reaches the feathers, is believed to indicate a high level of motivation. However, the performance of a vacuum activity itself may be a substitute for the real thing (e.g. Lindberg and Nicol 1997), so must be used with caution as a welfare indicator.

One response to long term confinement in a small pen is reduced activity and unresponsiveness, possibly associated with self-narcotisation using endogenous opiates (Broom 1988). Behavioural inhibition is commonly the response of cats to a shelter environment and may be easily missed unless detailed observations are made (McCune 1992).

Stereotypies (fixed sequences of behaviour performed over and over again with no obvious function) are also used as indicators of poor welfare. Though their exact cause and significance is debated (see Mason 1991), some may be signs of brain pathology (Dantzer 1986, Mason 1991). They are generally shown during stressful conditions, but may continue even when conditions are improved. Although they are a sign of current poor welfare, they may be a sign of past stressors rather than current ones. As with physiological measures the above must be interpreted with caution. For example, different measures might not co-vary (Mason and Mendl 1993). For example, Baxter and Plowman (2001) introduced coarse meadow hay into the diet of giraffes (*Giraffa camelopardalis*) in an attempt to decrease oral stereotypies due to lack of foraging or appetitive behaviour. Although the time spent feeding did not increase, the time spent ruminating did and there was a reduction in the time spent performing oral stereotypies.

1.3.4 Choices

All of the measures above (including immunological and physiological measures) are used as measures of poor welfare, but rarely as measures of good welfare (though different husbandry systems and so on may be compared to each other). Since the 'stress response' identifies arousal and cannot tell us whether the animal is finding the situation unpleasant, this makes physiological pleasure detection difficult. Behavioural signals which say 'I am happy' may signal to others that the individual is in possession of some valued resource, such as a food item, which the others may usurp. This would logically be selected against by natural selection in many cases (Broom, 1988). An animal's choices can solve this – preference tests work on the reasonable assumption that an animal given a choice between two or more options will pick the one that it prefers, though this may be hard to apply in a shelter setting.

1.3.5 Individuality

One possible problem with any animal experiment is that there is large variation between animals. This 'individuality' reduces statistical power when populations are under consideration (Martin and Kraemer 1987) but may be of interest in itself.

Individuality is particularly important to the study of animal welfare – if some animals find a particular enrichment aversive but the majority very much prefer it, results from the minority may be treated as 'error' and the enrichment recommended. This will lead to a significant reduction in welfare for the minority. Manteca and Deag (1993) also point out that individual differences in temperament are particularly important for the study of animal welfare, for the welfare of an individual largely depends on whether it can cope with environmental challenge. For example, Smith and Dobson (2001) also found heterogeneous responses of plasma cortisol in sheep during road transport. These highlight the need to study how the welfare of each animal in a situation is affected by individual differences in the perception and/or the response to a situation.

1.4 Welfare of cats in shelters / Behavioural techniques for measuring welfare

As mentioned in the introduction, the welfare of cats in shelters may be poor, even though the overall aim of the shelter is to improve their long-term welfare by rehoming. This section will first introduce standard shelter practice as observed by the author, review the research conducted on cat welfare, then cover environmental enrichment in theory and practice.

In the UK, the recommended minimum floor area for cats in laboratories is 0.5 m^2 per cat for individually housed cats under 3kg, and 0.75 m^2 for cats above 3kg. For group housed cats, this is reduced to 0.33 m^2 and 0.5 m^2 respectively (McCune 1999). While some shelters may keep cats in cages of around this size, most provide more space per cat, housing cats in relatively spacious group pens with an indoor and outdoor section (see Smith 1990), or singly or in pairs in smaller pens. These often consist of an inside pen connected by a cat flap to an outside pen, offering at least $4m^2$ of floor space. Pens are generally cleaned every day and proprietary tinned or dry cat food fed twice a day. Water is available *ad lib.* Due to time pressures on staff, cats frequently receive little social contact.

1.4.1 Behaviour of confined cats

Podberscek *et al.* (1991) investigated the behaviour of male laboratory cats. They found that cats rested and sat alone in 85 and 90% of observations respectively. The average percentage distribution of the other behaviours observed in the cats is shown in Fig 1.2. In shelters, stressed cats often perform behaviours such as eating, defaecating and escape behaviours when staff are not present, i.e. at night (pers. obs.), though Rochlitz *et al.* (1998a) report that cats are inactive during most of the night.

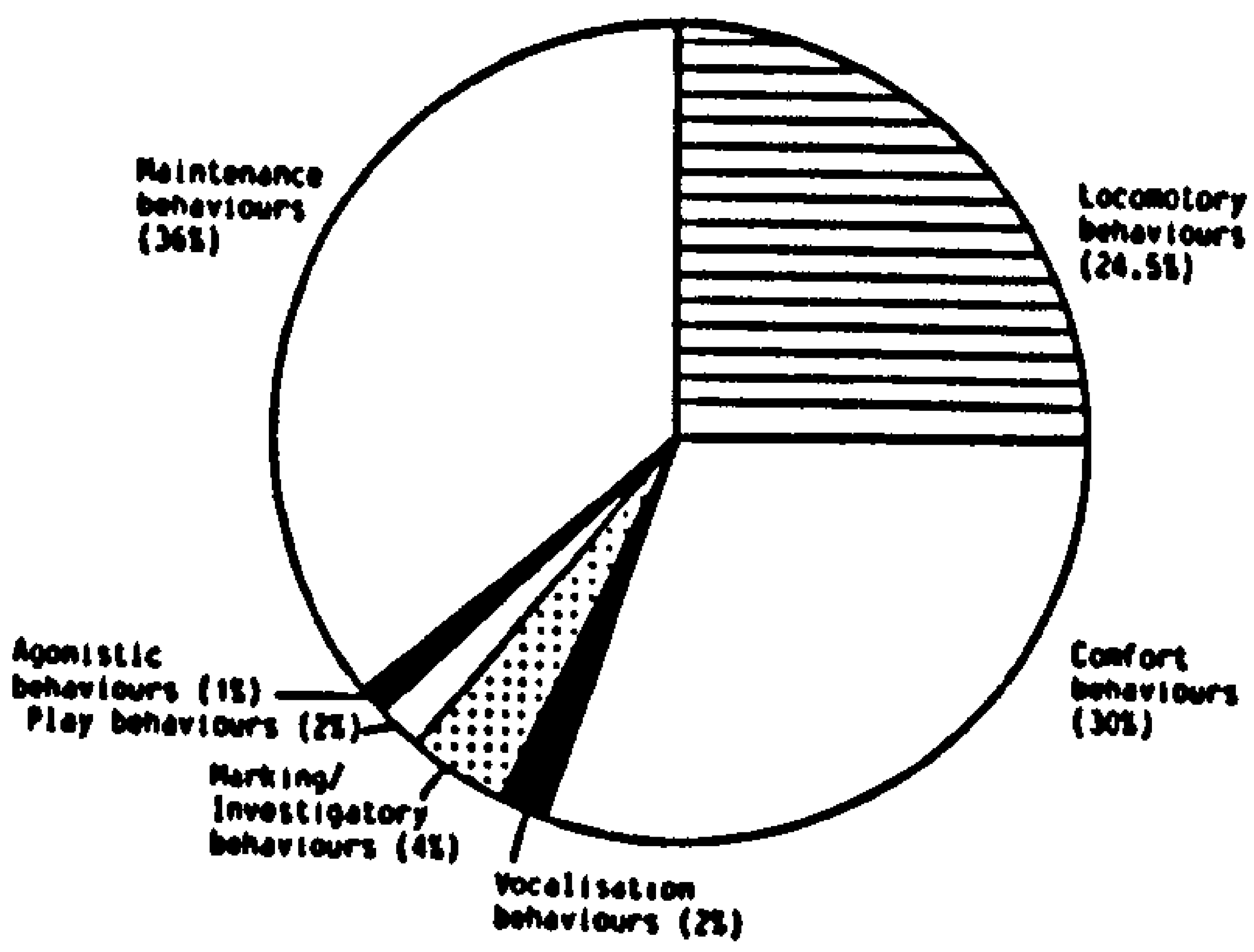


Figure 1.2 Podberscek *et al.*, 1991.
The distribution of the average percentage behaviours in the colony cats, not including resting and sitting alone (85 and 90% of observations respectively).
Maintenance behaviours: Sitting, eating, drinking, defaecating, and urinating.
Locomotory behaviours: Walking, running, and jumping.
Comfort behaviours: Grooming, scratching, sneezing, coughing, head shaking, stretching, and yawning.
Marking / investigatory behaviours: Clawing, scratching post, rubbing cage, anal sniff, body sniff, and wall / floor licking.

Stress decreases with time

Being placed into a shelter environment is known to be stressful for cats (see section 1). This abates with time as most cats adapt to the shelter environment. Rochlitz *et al.* (1998a, b) investigated the effects of quarantine housing on cats. They found that compared with the first day, the cats' cortisol to creatinine ratios (creatinine is used to calibrate cortisol with urine concentration) were significantly lower during and after the second month in quarantine, and that the ratio on the first day was so high as to be outside the normal range. The cats spent most of the first two weeks hidden in an opaque box on the floor of their cage. By the end of their first month, they spent less time hidden and more time sleeping and grooming.

After quarantine, owners judged that their cats were more affectionate, friendly and vocalized more than before quarantine. This was interpreted as cats re-establishing their relationship with their owners. Reported increases in timidity and nervousness may have been due to a reduced ability to cope with complex conditions after isolation in a barren, unchanging environment (see Broom and Johnson 1993).

Kessler and Turner (1997) studied boarding cats using a cat-stress-score (CSS, based on the cat assessment score of McCune 1992, 1994). The score describes seven possible stress levels based upon postural and behavioural elements. They found that the levels of stress declined over the two weeks of their study, but did not reach the stress levels of the control group by the end of the second week. In the second week, the average stress level of a third of the cats was higher than 'weakly tense' with 4% of cats rated higher than 'very tense'.

McCobb *et al.* (2005) studied 120 cats in 4 rescue shelters using the CSS and found a drop over time, however this result appeared to be largely based on data from a few outliers. Based on lack of correlation with urinary cortisol (see section 1.5.1), they concluded that the CSS was not a useful instrument for measuring stress, though acknowledged that the lack of correlation could be due to their inability to control for the highly variable housing conditions for individual cats in their study – the CSS is dependent on environmental conditions at the time of evaluation.

Other cats can increase or decrease stress

Kessler and Turner (1999a) found that cats which were not socialized to conspecifics were more stressed than socialized cats when put into group housing, and made the other cats in the group more stressed also. They concluded that cats which are not socialised to other cats should not be group housed, and recommended both single- and group housing equally for cats socialised to other cats. They also (1999b) investigated the effects of density and cage size, and found that group density was strongly and positively correlated with stress levels.

Although cats form stable social groupings in appropriate conditions (Liberg *et al.* 2000) enforced social contact can be a potent stressor (even for cats socialised to other cats) as cats are unable to join or leave the group. Durman (1991) and Smith *et al.* (1994) found that immediately following introduction to a group, individuals showed high levels of vocalisation and escape behaviour relative to individuals that had been confined for several weeks. The frequency of these behaviours lowered rapidly after the first four days. Cats present in the group for over a year performed more affiliative behaviours than cats present for less than a year. This suggests that familiar cats can provide a rich environment, though such relationships can take a year to form – little affiliative behaviour was observed between cats that had been present for less than a year. Studies by van den Bos (1998) and Bernstein and Strack (1995) also indicate that aggression is not a major stressor for stable colonies. However, long-term care of cats is not the aim of most shelters.

Van den Bos and de cock Buning (1994) studied a group of 10 female laboratory cats and found a linear rank order, with higher-ranking cats displaying more offensive threats, and a tendency to gain weight. Lower ranking cats displayed more defensive threats and tended to lose weight. This may have been due to the higher ranking cats spending most time on the floor, where the food was, while lowest ranking cats spent little time on the floor and used their resting sites for urination and defaecation. In light of this, Rochlitz (2000) recommends that feeding, resting and elimination areas should be provided at a number of different sites to reduce monopolization.

Relatedness of cats also affects how they interact. Bradshaw and Hall (1999) found that, when combined in a cattery, pairs of littermates were more affiliative to one another than pairs of unrelated cats (all pairs had lived together for at least a year).

Humans can increase or decrease stress

Carlstead *et al.* (1993) subjected individually caged laboratory cats ('stressed' group) to an unpredictable husbandry regime, consisting of irregular feeding and cleaning times, an absence of talking or petting by humans, and daily unpredictable manipulations. Control cats were maintained under the standard caretaking schedule. They found that the 'stressed' group were chronically stressed by the regime, with elevated urinary cortisol concentrations, increased response to ACTH challenge and increased time spent awake/alert and attempting to hide. They concluded that the unpredictable nature of human-controlled events relevant to the cats was the most stressful aspect of the regime.

Humans can cause stress even if the husbandry system is good, especially for cats unsocialised to humans. Using their cat-stress-score, Kessler and Turner (1999a) found that shelter cats which were unsocialised to humans were more stressed than cats socialised to humans, and recommended that cats unsocialised to humans should not be housed in shelters if possible.

Other work with dogs (*Canis familiaris*)(Hennessy *et al.* 1998) and pigs (Pedersen *et al.* 1998) has shown that positive human interaction can reduce stress in these animals in certain situations (venipuncture in dogs, tether-stalled housing practices in pigs).

McCune (1992) found that cats which had been socialised to humans as kittens were more friendly than unsocialised cats. Socialised cats adapted better overall to the stress of caging than unsocialised cats. Cats with timid temperaments, restricted experience, and of a young age were more likely to have problems adjusting to confinement and responding to novelty.

1.4.2 Environmental enrichment

Because mammals such as the cat rely on collecting and analyzing information for their survival, they have a psychological need for a high input of information (Poole, 1992). While most guidelines for housing specify only minimum cage/pen sizes, this is only significant in relation to the constraints it places on the animal's normal activities: what is more significant are the facilities the cage provides for the animal to carry out a range of behaviours (Poole and Dawkins 1999). Particularly important for confined animals which have their basic needs met is the possibility of carrying out behaviours that can expand to fill the available time (Hughes and Duncan 1988). Although an animal is by definition always doing something, behaviour such as foraging may be preferable to an unnatural amount of time spent resting.

Environmental enrichment has been defined as 'an improvement in the biological functioning of captive animals resulting from modifications to their environment' (Newberry 1995). I wish to clarify that in this thesis, good welfare will be considered a component of improved biological functioning. Environmental enrichment for mammals consists of providing them with a secure base and, when foraging, information to satisfy 'food for thought' (Poole and Dawkins 1999). Environmental features must be provided so that the animal is not in a state of constant fear. For shelter cats which are frequently housed in small pens with large windows overlooked by the public and other cats, this may take the form of having somewhere to hide in.

One way to increase mental stimulation is to increase the complexity of the environment. Work with primates suggests that objects with higher complexity are preferred (Sambrook and Buchanan-Smith 1997). Object preferences will also be affected by the species' behavioural ecology. Domestic cats are semi-arboreal and prefer high cages with raised platforms on which they can rest. Other animals can be thought of as highly complex objects because they interact and can be unpredictable, though see section 1.4.1 for remarks on cat-cat interactions.

In addition to complexity, controllability is also important. Control (or perceived control) of objects and events is important to animals "...this [desire for control] should not surprise us since control is *the* adaptive aspect of behaviour: control over what you eat, what eats you..., with whom you mate, etc." (Sambrook and Buchanan-Smith 1997). Beerda *et al.* (2000) found that stimuli which could not be predicted by dogs, such as sound blasts operated from outside an experimental room, induced saliva cortisol responses and a very low posture. Stimuli administered by an experimenter visible to the dog did not change cortisol levels and induced only moderate lowering of posture. Weiss (1972) found that rats which could predict and to some extent control electric shocks had a lower stress response than rats which received exactly the same shocks but with no opportunity to predict or control them. At the other extreme, total control cannot be automatically expected to relieve boredom. For example, Markowitz and Line (1989) studied enrichment of macaques by allowing them to press a lever to obtain food rewards. One macaque pressed a switch 130,000 times in a week, which common sense suggests must be incompatible with a macaque's normal activity budget.

Many mammals may spend some time investigating and manipulating novel objects. There are many anecdotal accounts about enrichments which increase competition and aggression when given to social groups of animals. Increasing the number of enrichments so there is no need for competition may solve these problems (Bloomsmith *et al.* 1988).

Novel stimuli are also valuable – many cats appear to enjoy looking outside through windows. De Luca and Kranda (1992) found that cats housed as a group in a room spent most of the day sitting on a window shelf, watching activity in the outside hallway. Since cats live in a different perceptual world to humans, some stimuli may be unintentionally aversive. For example, ultrasound generated from squeaky trolley wheels, television and computer monitors may be a cause for concern (Sales *et al.* 1988). Olfactory and auditory cues from, e.g. dogs in a neighbouring building, may be a cause of stress - McCobb *et al.* (2005) grouped cats in 4 shelters into 'high dog exposure' and 'low dog exposure' and found that the former scored higher in a measure of physiological stress (urinary CC ratio, see section 1.5.1). Many shelters play music for the benefit of cats, partly as a mask for other noises such as neighbouring dogs, partly for stimulation and socialisation to different voices. However, shelters may be noisy without the addition of music. In such cases, continual noise and stimulation may do more harm than good, especially since the cats have no control over the sound (Newberry 1995). Experimental results have been similarly mixed: Ogden *et al.* (1994) played recordings of tropical rainforest sounds to captive lowland gorillas (*Gorilla gorilla gorilla*). They found that the adults responded with increased locomotion, which was interpreted as a negative effect indicating agitation, while infants clung less, interpreted as a positive effect of the sounds masking other noises.

Determining whether an enrichment object is in fact increasing the animal's welfare can be difficult. It is generally assumed that a more varied or 'natural' behavioural repertoire increases welfare, as does a decrease in abnormal behaviours. However, for domesticated animals, both natural and artificial selection has altered their behaviour from that of their wild ancestors, increasing the complexity of interpreting changes in behaviour following enrichment. Most studies show that enrichment does not change baseline cortisol but does cause a lowered cortisol response to some other, acutely stressful situations (Carlstead and Shepherdson 2000). For example, sows exhibit increased cortisol secretion when piglets are weaned, but sows in large pens with straw show a significantly lower increase than sows in farrowing crates with no straw

(Cronin *et al.* 1991). Another approach is to use preference tests to see how much the animal values the object (see below).

Environmental enrichment for cats

Smith (1990) and Smith *et al.* (1994) showed that group housed cats used structures such as logs and chairs more often than the floor of the pens. Those at some height above the ground were preferred over those close to the ground. Wooden chairs were most clearly preferred, as they allowed cats to rest alone or with others, above the floor of the enclosure. Research by Roy (1992) and Smith *et al.* (1994) indicated that cats preferred resting places with a clear view of other cats. Rochlitz (2000b) recommended that cages should contain structures such as climbing frames, raised shelves, and platforms at different heights. Slanting boards will allow small or old cats to reach these areas. Loveridge (1994) and Loveridge *et al.* (1995) give an example of cat housing intended to meet cats' behavioural needs. However, any changes in husbandry must be balanced out against costs and the practicalities of cleaning out the enclosures.

Newberry (1995) points out that a common shortcoming of attempted enrichment is that the introduction of stimuli such as toys or music often has little functional relevance to the animals. Bradshaw *et al.* (1997) found that object play in cats may be motivationally similar to predatory behaviour. Play with objects may therefore serve as an enrichment for indoor cats that allows them to perform an element of their 'natural' behavioural repertoire. Hall (1995) found that the most intensive play was elicited by toys of small size (7x5x1 cm), which were moving, and covered in real fur, feathers, or fake fur. Play stops primarily due to increasing habituation to unchanging toy stimuli, and only slightly due to a general decrease in play motivation – play inhibition can often be overcome by presenting a toy with contrasting stimuli.

De Monte and Le Pape (1997) found an important novelty effect of new objects for single-housed adult laboratory cats. Introduction of objects (a log tied to the side of the cage and a ball suspended from the ceiling) into cages resulted in a decrease in inactivity and self-play activities, and an increase in sniffing objects and play behaviour with objects. The log elicited mainly rubbing and paddling behaviours while the ball (a moveable object) especially stimulated play.

Putting dry cat food into containers through which the cat has to extract individual pieces, or plastic bottles with small holes in so that food drops out when they are rolled around (McCune 1995 and pers. obs.), elicit manipulative behaviours and encourage play / predatory behaviour even when the novelty wears off. This is presumably due to the reward value of the food obtained.

Preference testing

What an animal perceives as best for its welfare should be reflected in its preferences. These can be measured directly, by allowing an animal to pick from a range of options, or indirectly by an animal making an operant response such as lever pressing to obtain a reward. For example, Sanotra *et al.* (1995) investigated the relative attractiveness to domestic chicks (*Gallus gallus domesticus*) of various substrates for pecking and dustbathing. Two alternatives at a time were offered, and the amount of pecking at / dustbathing on each recorded.

1.4.3 Individual variation between cats

As mentioned in section 3.5, individual variation can cause problems for welfare experiments. An example is the work of Carlstead *et al.* (1992) who subjected 8 cats to a stressful regimen (which included being bled by jugular venipuncture). Although there was no overall significant effect of stress, four of the stressed cats had elevated urinary cortisol compared to baseline, and four had decreased cortisol concentrations. The latter may have been due to the rewarding properties of being held during blood sampling, as these four cats were the most tractable and affiliative with people.

Feaver *et al.* (1986) investigated methods for rating the individual distinctiveness of cats. Two observers watched fourteen adult domestic cats for 3 months, and showed that observer's ratings of an individual's distinctiveness were frequently reliable.

These ratings were compared with results of direct recording methods, where suitable measures were available, and in five out of six cases they were significantly correlated. On the basis of this study and others (summarised in Karsh and Turner 1988), cats may be separated into three main types: active and aggressive cats, timid and nervous cats, and sociable and confident cats. Such a factor may influence reactions to stress – McCune (1994) identified two styles of response to caging: active cats which will be mobile, vocalize loudly and may destroy cage furniture; and passive cats which will be immobile, tense and unresponsive. Active cats will be more easily identified as having poor welfare, though the welfare of passive responders may be as poor.

Such ratings provide information on subtle aspects of an animal's behaviour that might otherwise be overlooked (Manteca and Deag, 1993), although the time required to score individuality in this way may limit use of the method. Feaver *et al.* also pointed out that a human's behavioural style sometimes does not change from one circumstance to the next, but in some circumstances will alter very considerably. Lowe and Bradshaw (2001) investigated the ontogeny of individuality in cats, and found that the degree of boldness, possibly coupled with an investigative element was consistent from 4 months to at least 2 years old. This may be similar to the shy/bold factor identified by McCune (1992).

1.5 Physiological techniques for measuring welfare

There have been few experiments which have used physiological measures as measures of cat welfare: the studies of Rochlitz *et al.* 1998a and Carlstead *et al.* 1993 which measured cortisol in urine have been discussed in section 1.41. Few other (non-glucocorticoid) physiological measurements have been made on cats relating to welfare, so the rest of this section will concentrate on measuring ACTH activity.

Glucocorticoid release into the blood is relatively fast. Carlstead *et al.* (1992) found that cats subject to ACTH challenge had serum concentrations of free cortisol rise tenfold within 10 minutes post-administration. Urine offers advantages as a non-invasive method of HPA assessment, as the blood collection procedure itself may elicit activation of corticosteroids. In addition, urine concentrations can be considered as more integrative than plasma concentrations, so may be more accurate for the detection of variations in the HPA axis and SNS basal activity (Hay and Mormède, 1998).

1.5.1 Urinary analysis

Carlstead *et al.* (1992) found that urinary cortisol increased twofold by 2 hours post-ACTH challenge, and was correlated with serum concentrations. Twenty-four hour urinary cortisol concentrations correlated with the serum cortisol response, showing that 24 hour urinary cortisol is a good index of plasma cortisol.

Carlstead *et al.* (1993) and Rochlitz *et al.* (1998a) used similar techniques to assess the adrenal responsiveness of cats to psychological stressors. They used double-tiered litter trays with the top tray perforated with small holes so that urine drained into the lower tray. The top layer contained non-absorbent litter granules which mimic the usual substrate for urination. A sample of urine was collected every 24 hours (no data to the author's knowledge has been collected for the cat, but human urinary cortisol is stable for at least 48 hours at room temperature, Kong *et al.* 1999). It was collected at the same time each morning (Rochlitz *et al.* filtered samples through muslin) and frozen at -20°C until assayed. Cortisol was measured using a radioimmunoassay (RIA) system. Carlstead *et al.* (1992) reported that assay sensitivity was 2.0 ng/ml, and the inter and intra assay coefficients of variation were 6.8% and 5.9% respectively.

The concentration of cortisol in urine is related to the concentration of creatinine (measured by spectrophotometry) to account for changes in fluid balance, the result expressed as the cortisol to creatinine ratio. Creatinine is widely used as an index of urine concentration as it remains relatively stable in the plasma of healthy animals, and can be readily measured in urine (Bahr *et al.* 1998).

Baseline values taken over several days (as conducted by Carlstead *et al.* 1992, 1993) are valuable as indications of variability of urinary cortisol from day to day in undisturbed animals, as well as reducing intra-individual and measurement error in the baseline mean (Martin and Kraemer 1987).

Radioimmunoassays work as follows (from Guyton and Hall 2000, though see also Selby 1999): first, an antibody that is highly specific for the hormone to be measured is produced. Second, a small quantity of antibody, the assay fluid (in this case urine) to be measured, and an appropriate amount of standard hormone tagged with a radioactive isotope are simultaneously mixed together. It is essential that there must be too little antibody to bind completely with both the tagged hormone and the hormone in the urine. They therefore compete for the binding sites of the antibody, and the quantity of each of the two hormones that binds is proportional to its concentration in the assay fluid. Third, after equilibrium binding has been reached, the antibody-hormone complex is separated from the solution, and the radioactivity in it counted, so the amount of tagged hormone can be assessed. If a large amount of radioactive hormone has bound with the antibody, there was only a small amount of hormone in the assay fluid to compete with it, and vice versa. Fourth, to make the assay quantitative, the procedure is performed with standard solution of untagged and tagged hormone at different proportions, and a standard curve plotted.

High-pressure liquid chromatography (HPLC) was previously used on cat urine (Carlstead *et al.* 1992) to determine the relative proportions of immunoreactive cortisol metabolites, and the contribution of immunoreactive cortisol to the RIA. Urine was combined with ^3H cortisol (to act as a tracer) before being run. Separate fractions of eluate were collected and assayed for radioimmunoactivity using the cortisol RIA. Cortisol immunoreactivity coeluted with the ^3H cortisol, and recovery of cortisol in fractions close to the ^3H cortisol elution was 93%.

Urinary catecholamine assessment may also be used (e.g. Hay and Mormède 1998, Hay *et al.* 2000) to provide information about the sympathoadrenal axis. To the author's knowledge no such work has been carried out on cats.

McCobb *et al.* (2005) determined urinary CC ratios for 97 cats at 4 shelters, and found almost no correlation between urinary CC and time spent at the shelter, however they looked at only one datum per cat rather than repeated measures data. They found no significant relationship between noise level in the shelter and stress level in cats as assessed by the CSS, or by urinary CC. They found that cats with high dog-exposure levels had significantly higher urine CC ratios than those with low exposure. There was very little correlation with CSS and the amount of time after admission, and none between CSS and urinary CC.

1.5.2 Faecal analysis

Faecal cortisol and its metabolites can also be used. Graham and Brown (1996) gave 3 cats intramuscular injections of radiolabelled cortisol and collected urine and faeces after spontaneous elimination. Most urinary radioactivity was detected in the first sample collected at 3.9 ± 2.5 h postinjection, and accounted for 14% of the total radioactivity recovered. Most faecal radioactivity was excreted at 22 ± 6 h. and constitutes the other 86% of radioactivity recovered. Schatz and Palme (2001) conducted a similar study, and found peak urinary radioactivity after 9 ± 3 h, and peak faecal radioactivity after 22 ± 6 h. Again, faecal metabolites were 82% of recovered radioactivity.

HPLC of urine by Graham and Brown detected four urinary metabolites (Fig. 1.3), one of which appeared to be cortisol – this accounted for only 1.9% of the total cortisol metabolites excreted. This was increased to 5% by enzyme hydrolysis to separate cortisol from cortisol binding protein (cortisol is transported in the blood bound to transcortin and, weakly, to albumin, with 5-10% being free (Bush 1991). HPLC of faeces detected several cortisol metabolites, none of which eluted with cortisol or corticosterone reference tracers, though two polar metabolites were quantifiable using a corticosterone RIA, due to cross reactivity (Fig. 1.4). Cross reactivity occurs when the antibody used binds with hormones other than the desired one, though at much lower affinities. HPLC on faeces was also performed by Schatz and Palme (2001), who also found small amounts of cortisol and large numbers of steroid metabolites.

Graham and Brown found that cats subject to an ACTH challenge had an increase in faecal cortisol metabolite concentrations 24-48 hours later, so adrenocortical activity can be monitored by measuring cortisol metabolites excreted in faeces. Schatz and Palme also subjected cats (n=10) to ACTH challenge and found peak metabolite concentrations 24-49 hours later (median 25). The delays represent intestinal passage time, with variations due to constipation. Schatz and Palme also measured metabolites following dexamethosone injection (which suppresses adrenocortical activity). Despite large variations, faecal metabolite concentrations tracked activity of the adrenal cortex well.

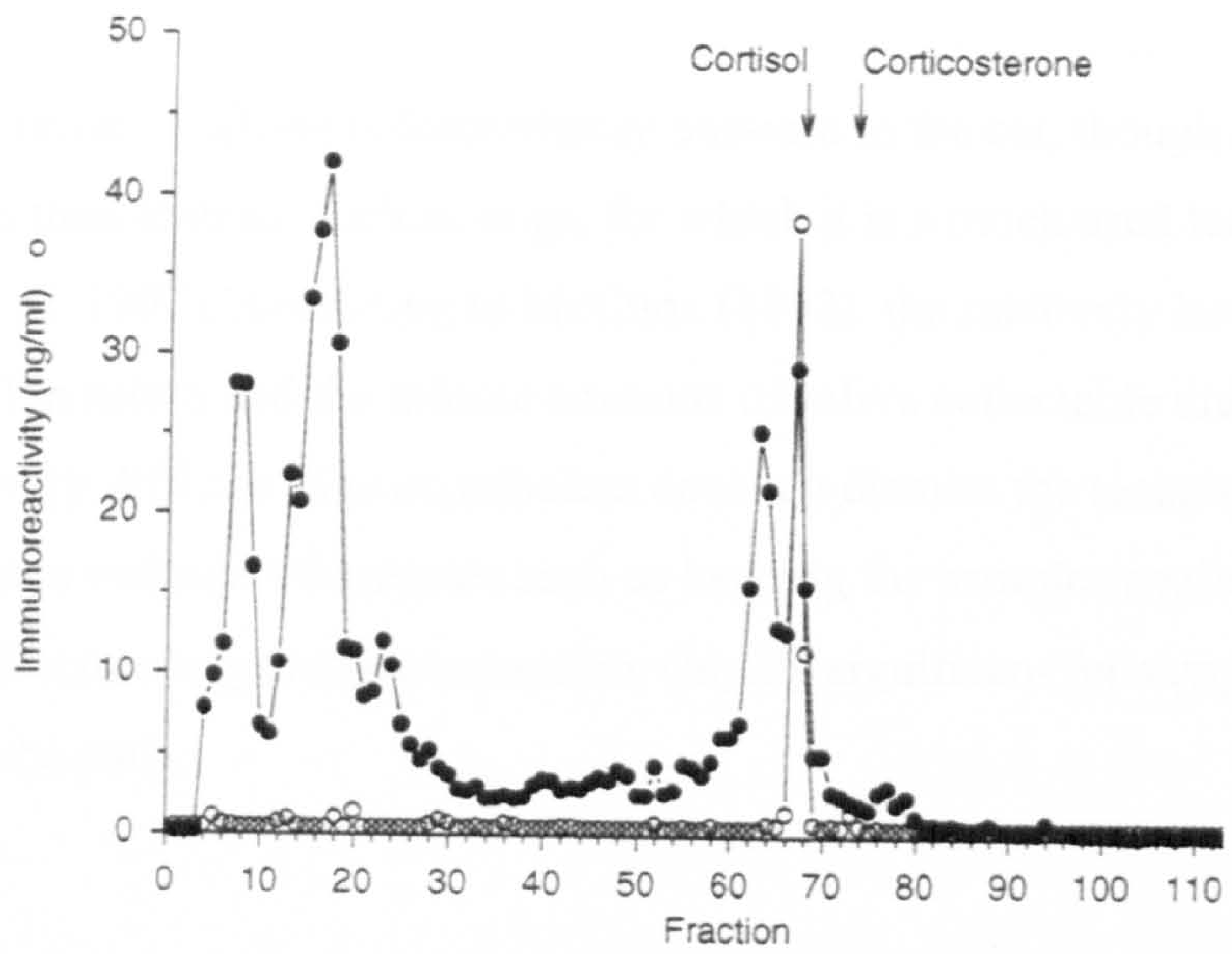


Figure 1.3
Urinary cortisol metabolites

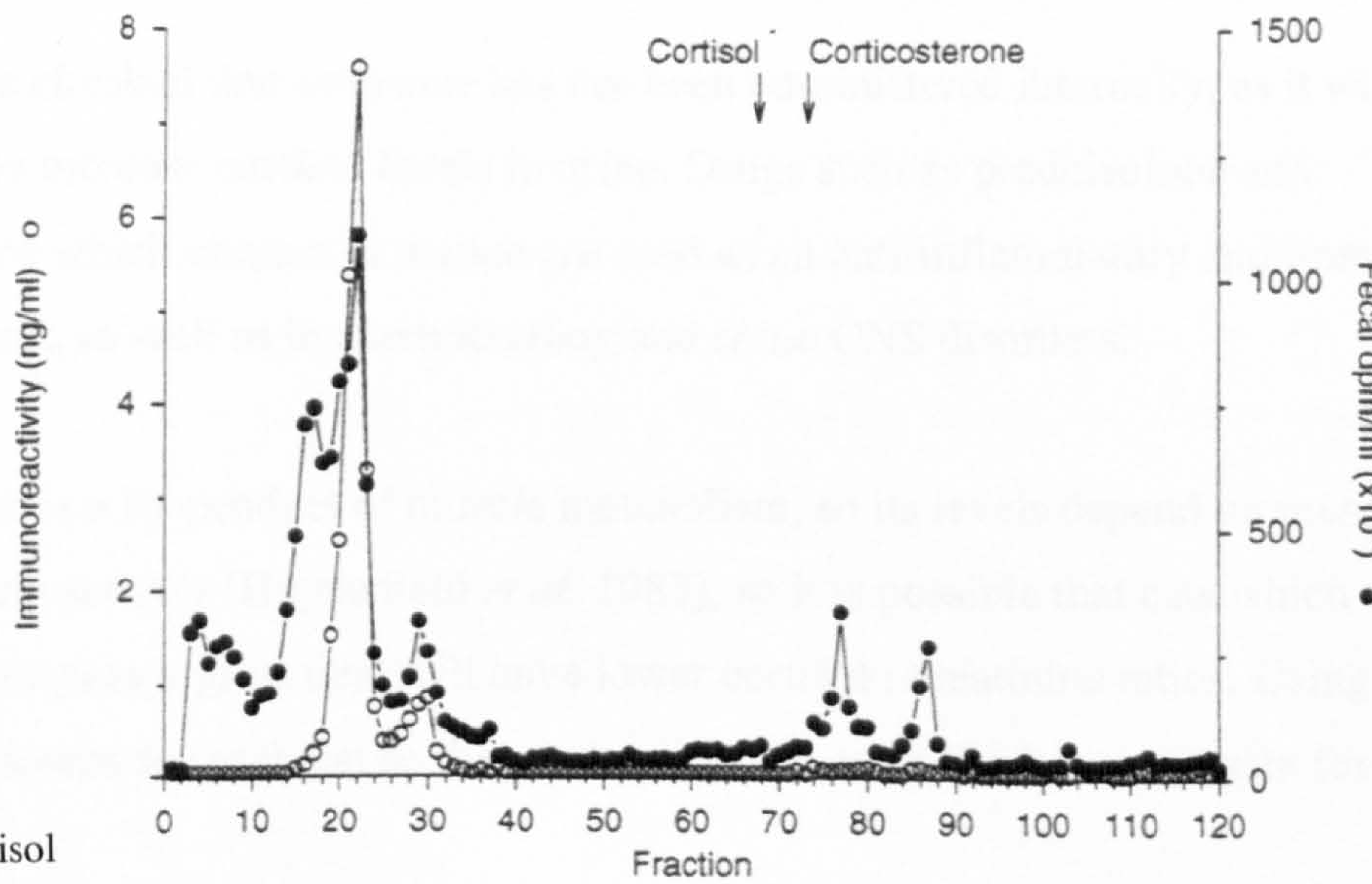


Figure 1.4
Faecal cortisol metabolites

Figs 1.3 and 1.4, Graham and Brown (1996)
HPLC separation of metabolized urinary (Fig 1.3) and faecal (Fig. 1.4) cortisol metabolites after i.m. injection of ^{3}H-cortisol. Imunoreactivity of each fraction was determined by a cortisol (Fig 1.3) or corticosterone (Fig 1.4) RIA. ^{3}H-cortisol and ^{3}H-corticosterone reference tracers shown. (No radioactivity scale for Fig 1.3 was given by Graham and Brown.)

1.5.3 Salivary analysis

Salivary cortisol analysis is theoretically possible in the cat, though cats produce far less saliva than animals such as dogs, for which it is a much used technique (e.g. Beerda *et al.* 1997). According to McCune (1992), the relatively low concentrations of cortisol in saliva and the minute amounts of saliva collectable make accurate assaying very difficult. She nonetheless does not dismiss the technique, and recommends various refinements such as keeping the samples cooled at all stages during collection to prevent evaporation (highly significant for samples of 40μl – 200μl as she had).

1.5.4 Problems with physiological techniques

Despite the well-documented efficacy of cortisol as a measure of adrenocortical activity, occurrences other than stress can affect its production and levels in urine.

It must be checked that cortisone has not been administered internally, as it will artificially increase cortisol levels in urine. Drugs such as prednisolone and prednisone which contain cortisone are used as an anti-inflammatory and immuno-suppressant, as well as in chemotherapy and some CNS disorders.

Creatinine is a by-product of muscle metabolism, so its levels depend on muscle mass and muscle activity (Heymsfield *et al.* 1983), so it is possible that cats which are well-muscled or pace a great deal will have lower cortisol : creatinine ratios. Using baseline scores for each cat so that each is its own control can compensate for this.

1.5.5 Variations in cortisol levels

It is not certain whether cats have a circadian rhythm of cortisol production (Sparkes *et al.* 1990) - Johnston and Mather (1979) found no circadian rhythm in a study of six cats, but Scott *et al.* (1979) reported a circadian rhythm in three of four cats studied under single housed laboratory conditions, with lower blood cortisol levels in the morning compared to the afternoon and evening. Individual variation has been shown to have a marked effect on the profile of circadian variations in humans (Brandenberger *et al.* 1973, in Hay and Mormède 1998).

Hay *et al.* (2000) found that there was a high correlation between the excretion rate of catecholamines and glucocorticoids over 24 h and their concentrations in urine produced during the night and early morning in pregnant pigs. Variations between sows were most prominent in nocturnal and early morning (2000-0900) urine. They also found that the cortisol: cortisone ratio changed during the day, from a maximum of 1.14 during the interval 0400-0800 to a nadir of 0.76 in the evening 1600-2000, though did not know how to account for this.

A blunted circadian rhythm for cortisol is often measured during situations of chronic stress in pigs, rodents, and humans (see de Jong *et al.* 2000). Pigs raised in a barren environment had blunted circadian rhythms, but lower overall salivary cortisol than enriched pigs, reflecting decreased welfare of barren-housed pigs. The circadian rhythm of salivary cortisol was measured at different ages in these pigs to eliminate the possibility that differences in baseline salivary cortisol simple represented a phase difference in circadian rhythm of HPA activity. This is always possible, especially if moving animals to a facility with different feeding times, and light / dark cycles, etc., for instance when cats are handed over to a shelter. The possibility can be eliminated if similar levels of cortisol at the same time of day are found over the first few days of the new regime. However, since stress levels are expected to change in the first few days after moving, particularly equable animals that show little stress from the move should be used for this test.

Further, in humans, serum cortisol levels are affected by variations in cortisol-binding protein (CBP) and do not correlate well with cortisol production rates unless differences in CBP are accounted for. Brennan *et al.* (2000) found no relationship between plasma and urinary levels of corticosterone in rats, possibly due to plasma levels of corticosterone-binding globulin being reduced after stress, or to the circadian rhythm in CBG binding capacity. However, urinary free cortisol levels in humans appear to be unaffected by CBG and correlate well with changes in cortisol production (Bright and Darmuan 1995). No similar studies on domestic cats are known to the author.

Sex differences may occur, too. Although no work known to the author has been conducted on domestic cats, work on dogs by Beerda *et al.* (1999b) found that females showed greater HPA responses (salivary and plasma cortisol) and stronger behavioural indications of acute stress than male dogs to acute stressors such as a sound blast and injected corticotropin releasing hormone. Garnier *et al.* (1990) followed dogs over the first twelve weeks after admittance to a veterinary hospital and found that females had higher plasma cortisol on day 2 after admission, but not in later tests after 5 and 12 weeks. However, no sex differences were found in a later study of urinary cortisol in dogs subject to differing degrees of chronic stress (Beerda *et al.* 2000). The sample sizes in this study were not large (the largest was N=24), and consisted of a mix of breeds (only 15 dogs were tested in Beerda *et al.* (1999b), all laboratory beagles). Van Vonderen *et al.* (1998) also found no sex effects in urinary cortisol from dogs suffering acute stressors, though the use of urinary cortisol might have blunted any acute effect. In conclusion, female dogs appear to be more susceptible to acute stressors, though it is not known if this extends to chronic stressors.

One of the Beerda *et al.* studies (1999b) above also found an effect of the animals' previous experiences - bad weather conditions during spacious outdoor housing induced early stress that attenuated the negative appraisal of a subsequent period of indoor social and spatial restriction. Dogs with pleasant weather during the outdoor housing had a greater increase in urinary cortisol and other behavioural measures indicating stress when undergoing the indoor restrictions than dogs accustomed to bad weather.

1.6 Conclusion

The welfare of many cats in shelters is poor, especially soon after admission (McCune 1992, Kessler and Turner 1997). Though environmental enrichment may offer ways to improve welfare (Smith *et al.* 1994, De Monte and Le Pape 1997), there is still scope for much research into this topic. Although most studies of cat welfare have been purely behavioural in nature, physiological measures such as urinary cortisol offer further validation and a less subjective measure of cat stress.

1.7 Aims of the thesis

The aims of the author's research were to look at how stress can affect the welfare of cats in rescue shelters, and investigate potential methods of alleviating stress and improving welfare. In particular, the following research questions were considered:

1) What are the cats' responses to admission to a rescue shelter, and how do they change over time?

2) How do these responses relate to each other? In particular, how do urinary cortisol:creatinine (CC) ratios and the Cat-Stress-Score (CSS) relate to each other and to other behavioural measures?

3) What effect does box provision have on these responses to being admitted?

4) What effect does increased social contact from a human have on these responses to being admitted?

5) How do these two treatments affect cat behaviour and urinary CC once cats are in the homing area of the cattery?

6) Is there any effect of box provision for long-stay cats which are in the homing area of the cattery?

7) Are box provision or increased social contact likely to affect the speed at which a cat is rehomed?

1.8 Structure of the thesis

Chapter 2 is a longitudinal study examining the responses of twenty-five cats to their first 8 days after admission to a rescue shelter, and their responses for the first seven days after being transferred to a different housing block on the same site. It investigates urinary CC ratios, Cat-Stress-Scores and other behavioural measures: both how they change over time, and how they relate to each other.

Chapter 3 examines the effects of two treatments: box provision, and increased social contact, in a two-factor ANOVA design over the first fortnight after admission.

Chapter 4 examines the effect of adding boxes to the housing of long-stay cats in the homing areas of three different catteries. This is a longitudinal study, with each cat acting as its own baseline.

Chapter 5 provides a discussion of the thesis' main findings. Guidelines to shelters are presented for minimising the stress of cats in rescue shelters, and the probable effects on rehoming.

Chapter 6 is a one-page summary of the thesis' main findings.

Chapter 2: Experiment 1 – Behavioural and physiological measures of stress in shelter cats

2.1 Introduction

As mentioned in the literature review, admission to an animal shelter is stressful for most cats due to the contrast between the social, temporal and spatial structure of the shelter and that experienced in the domestic environment (Bradshaw 1992, McCune 1992, Kessler & Turner 1997). Symptoms of stress may gradually disappear as the cat learns about, and adjusts to, its new surroundings. To what extent this ever occurs and over what time it takes place depends, amongst other things, on the temperament of the animal, its previous experience of shelters (McCune 1992, 1995) and on the housing conditions (Smith *et al* 1990; Roy 1992; Kessler & Turner 1999a, 1999b).

In a cat shelter, the process of adjustment can take from 2-5 weeks, though this shows much individual variation (Smith *et al* 1990; Kessler & Turner 1997; Rochlitz *et al* 1998a). Though there has been much work on the behavioural changes that take place as cats adapt to shelter life (Smith *et al* 1990, McCune 1992; Kessler and Turner 1997, 1999a, 1999b), there have been relatively few studies incorporating both behavioural and physiological methods (Carlstead *et al* 1993, Rochlitz *et al* 1998a). Carlstead *et al* (1993) experimentally stressed laboratory cats, looking at urinary cortisol to creatinine (CC) ratios and behavioural measures such as hiding and the time spent alert. Rochlitz *et al* (1998a) studied cats housed in a quarantine cattery, also looking at urinary CC ratios and behavioural measures. Both authors found broad correspondence between cortisol and behaviour.

No other work investigating CC ratios at a shelter had been published at the time of the study. Though McCune's Cat Assessment Score and Kessler and Turner's CSS had been successfully used by their authors (McCune 1992, 1994; Kessler & Turner 1997, 1999a, 1999b) and in numerous student projects (e.g. Kakuma and Bradshaw 2001) in shelter settings, and are fast becoming the benchmark for cat stress studies, no validation of the score had been published since McCune (1992). In 2005, McCobb *et al.* studied urinary CC and CSS in a cross-sectional study, and found a very low correlation between urinary CC and time since admission, no correlation between CSS and time spent at the shelter, and no correlation between CC and CSS. This may have been due to their measuring only one datum per cat, rather than time course data, and other methodological problems such as pooling data from 4 different shelters.

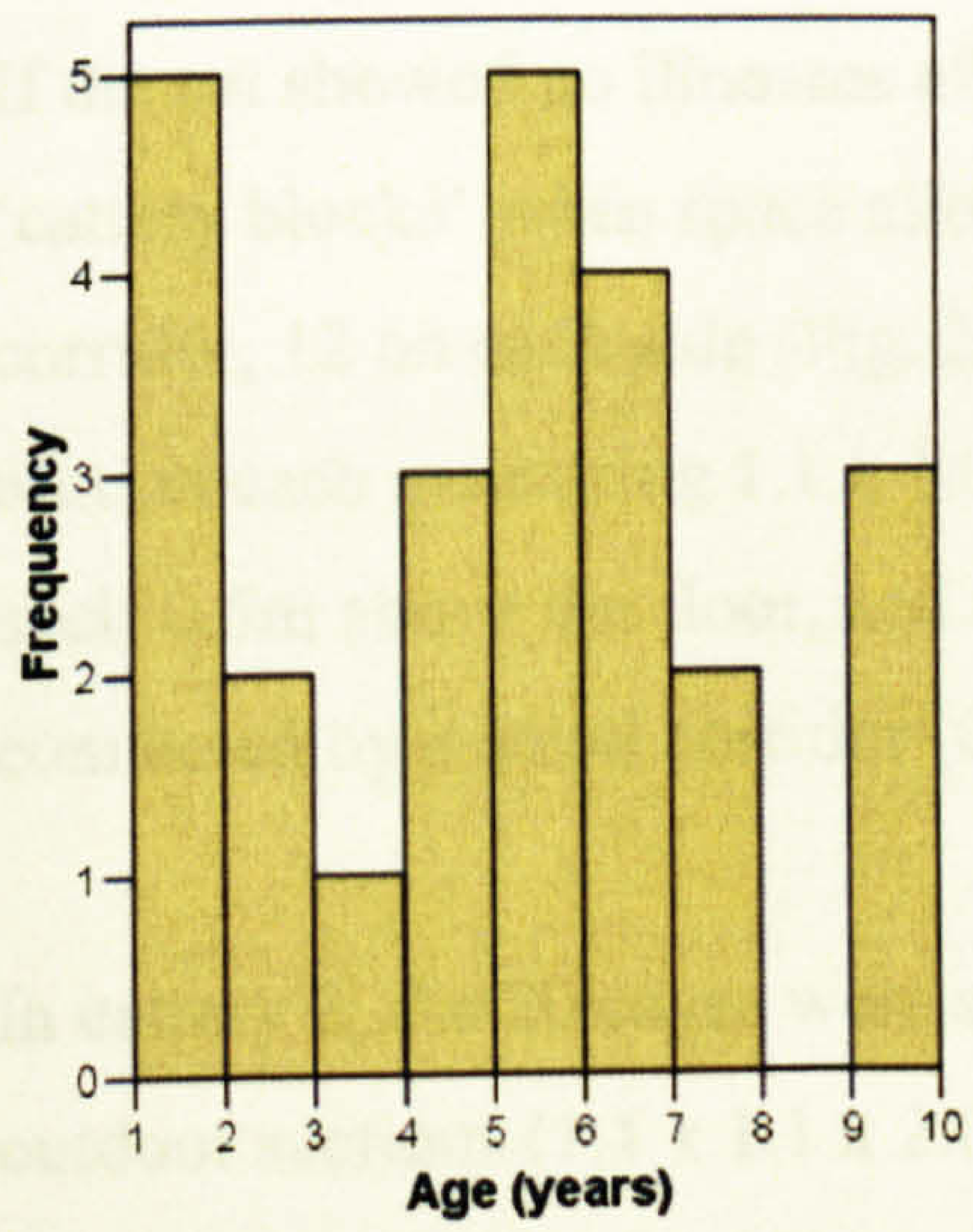
This study aims to add to the growing literature on shelter cat stress with a detailed assessment of adaptation to cat shelters, investigating CC ratios, CSS and other behavioural measures.

2.2 Methods

2.2.1 Subjects

Twenty-five boarding cats (age 1-10 years, Figure 2.1; 12 female, 13 male; 23 owned, 2 stray; 2 entire (both male), 23 neuter) were observed during their stay in a rescue shelter (Blue Cross adoption centre, Southampton, UK). All cats were in good condition and free of serious illness during their observation. All cats except two of the males were neutered, and all cats had been given up by their owners, except one male and a female which were strays. The range of sexes, ages, and neuter status were representative of the shelter as a whole. Strays were slightly under-represented in the sample, as many of these cats were excluded from the study due to malnourishment, illness, or their owners swiftly reclaiming them. The study was carried out from late November 2001 to March 2002.

Figure 2.1 Histogram of age of cats observed at the Blue Cross shelter



2.2.2 Housing

Upon entering the shelter, cats were housed in an 'admissions block' (Fig 2.2a), consisting of 18 indoor single cages measuring 1.2x0.7x0.7m (WxDxH). The inside surfaces were opaque white plastic, and the front was a grille through which the occupant could see and be seen by cats in cages on the other side of the room (Fig. 2.2b). Each cage was furnished with food and water bowls, a litter tray, blankets and a cut away box for hiding behind. Toys were provided if the cat showed a propensity for play. The public were not generally allowed into this block.

Figure 2.2a (Left) View down admissions corridor.

Figure 2.2b (Right) Typical cage in admissions block

If the cat showed no illnesses after a week or so, it was transferred to one of two 'cattery blocks' when space allowed. Cattery 1 had 24 cages either side of a central corridor, 12 on each side (Fig. 2.3a). Each cage consisted of an indoor and an outdoor section each measuring 1.1 x 1.0 x 2.1m. Both indoor and outdoor sections had a large shelf 0.5m above the floor, and another shelf 1.5m above (Fig. 2.3b). They were connected by a small corridor (0.2 x 0.3 x 0.2m) at floor level with a cat-flap.

In cattery 2, the 20 cages were similar to cattery 1 and consisted of indoor and outdoor sections (1.1 x 1.1 x 2.0m) connected by a passage 0.3 x 0.2 x 0.3m. There was a small shelf 0.4m off the floor, a large shelf 0.6m above, and two further small shelves at 1.0m and 1.5m (Fig. 2.4). Each was furnished the same as cattery 1 except there was no cat bed. Due to the layout of the catteries, cattery 2 was generally quieter than cattery 1.

In the indoor section of both catteries, there was a water bowl and litter tray at floor level. On the large shelf was a cat bed with blanket (cattery 1 only), a cut away box with blanket and a food bowl. The public was allowed into these two blocks from 1000-1530. Occasionally, cats which were considered particularly stressed were housed in a separate room in the admissions block in cages identical to those in cattery two, though the public was generally not allowed access to them.

Figure 2.3a (Left) View down cattery 1 corridor
Figure 2.3b (Right) Typical cage in cattery 1 (outside portion – inside portion identical, reached by cat-flap on ground level, shown at the bottom of the picture)

Figure 2.4 Typical cage in cattery 2 (inside portion – outside portion identical, reached by cat-flap at ground level).

In all three blocks, staff entered at 0830, fed the cats, cleaned the cages and performed other caretaking duties. This was generally completed by 1000, when the public was allowed to visit the cattery blocks. During the day, staff spent at least 5 minutes with each cat, stroking, grooming or playing with it according to its preference. The shelter was closed to the public at 1500, the cats were fed again at around 1515 and the shelter was shut at 1645. Though one member of staff was in charge of each block, various other members of staff worked there to cover for them on their days off.

2.2.3 Physiological measures

Urinary cortisol was measured using a similar technique to Carlstead *et al.* (1993) and Rochlitz *et al.* (1998a). Urine samples were collected daily between 0830 and 0930 using two plastic litter trays, one tray being placed within the other. The upper tray was perforated with small holes and contained small oval polypropylene granules 5 x 3 x 3mm, so that urine drained through to the lower tray. The same tray was left in the cage for the whole 24 hours. Faeces were removed by the author within an hour of deposition. Urine samples of 3-5ml were chilled in aliquots at 1°C for 9 hours, then transferred to –20°C until assayed. This collected urine represented urine produced since 0830 the previous day, and the data were interpreted accordingly. For assay, the thawed urine was centrifuged at 30,000 rpm for 5 minutes. The supernatant was used for analysis. Urinary cortisol was measured by the author using the ELISA method at the Chester College Centre for Stress Research (Chester College, Chester, UK).

The ELISAs worked as follows: first, anti-cortisol antibodies are adsorbed onto the surface of a 96 well ELISA plate. The sample is then mixed with enzyme labelled cortisol and added to the wells. The sample and labelled cortisol compete for binding to the anti-cortisol antibodies. The plate is then washed to remove unbound molecules. This leaves only the bound sample cortisol and labelled cortisol, in proportion to concentration in the mixture. The labelled cortisol remaining bound to the plate can then be quantified by adding the enzyme substrate and measuring the (coloured) product using a spectrophotometer. The colour change observed is inversely proportional to the amount of cortisol in the sample, which can be quantified by using a standard curve of known cortisol concentrations.

Validation of ELISA for Urinary Cortisol

A dilution series of cat urine resulted in a displacement curve parallel to the cortisol standard curve. Recovery of cortisol (1.25ng/ml) added to diluted urine was 104%, coefficient of variation (CV) = 4.3% (n = 3). Recovery of cortisol (25ng/ml) added to urine then diluted down was 104%, CV = 14.3% (this is higher due to the dilution steps compounding any errors introduced in the spiking step). The intra-assay CV was 5.70% and the overall inter-assay CV was 9.82%. To reduce this, assays conducted on samples from the same cat were conducted together where possible or matched according to a control sample if not. This reduced the inter-assay CV for each cat's samples to an average of 3.31% (highest CV=6.02%). The inter-assay CV for creatinine was 3.96%.

2.2.4 Behavioural measures

Three sets of behavioural measures were taken: CSS, approach test, and coded measures. The observations were carried out as shown in the schedule (Table 2.1).

The order cats were observed was fully randomized, with the condition that all cats in a block (admission, cattery1, cattery2) were tested contiguously. With this procedure a maximum of eight cats could be studied at a time. Cats were observed daily from admission to the shelter until a fortnight after entry to the cattery block, though nearly all cats were rehomed before then. In addition, every hour, beginning at 0830 and ending at 1630, each cat was video recorded for 30 seconds, to be later analysed for data such as position, behaviour, etc. These data were unfortunately lost before analysis.

Table 2.1 Observational schedule

Time	Test
0830	Check overnight coded measures
0845	Collect urine samples, replace litter trays
0930	Check morning coded measures
1040	CSS
1140	Approach test
1240	CSS
1340	CSS
1440	Approach test
1540	CSS, check coded measures

2.2.5 CSS

The Cat-Stress-Score (CSS) was developed by Kessler and Turner (1997), based on McCune's Cat Assessment Score (1994). The CSS describes seven possible stress levels of a cat based upon postural and behavioural elements (Table 2.2). The scores range from 'fully relaxed' (score 1) to 'terrorized' (score 7). All scoring in this study was done by the author (male, 24 years). The CSS was assessed after an initial one minute of observation in front of the cage. If the cat had a member of staff in the cage, or was otherwise disturbed (such as by members of the public) immediately before or during the test, the author carried on observing the remaining cats and returned to the cat later. Kessler and Turner remark that the CSS can be applied in all housing forms, but not when temperatures drop below 15°C, as the cats do not assume a relaxed position in the cold. Due to a heating fault, temperatures in one of the cattery blocks fell below 15°C during January, which made differentiating between scores 1-3 more difficult, though not impossible. Many elements are not affected by the cold such as eyes, pupils, whiskers and ears, and the observer can make allowances for cats being more tightly curled, etc. From the author's experience this does not decrease reliability when scoring the CSS, though it was felt that the scoring may reflect the cat's internal state less accurately.

Table 2.2 CSS scoring (from Kessler and Turner 1997)

Score	Body	Belly	Legs	Tail	Head	Eyes	Pupils	Ears	Whiskers	Vocalisation	Activity
1 Fully relaxed	*i:* laid out on side or on back *a:* not applicable	exposed, slow ventilation	*i:* fully extended *a:* not applicable	*i:* extended or loosely wrapped *a:* not applicable	laid on the surface with chin upwards or on the surface	closed or half opened, may be blinking slowly	normal	half back (normal)	lateral (normal)	none	sleeping or resting
2 Weakly relaxed	*i:* laid ventrally or half on side or sitting *a:* standing or moving, back horizontal	exposed or not exposed, slow or normal ventilation	*i:* bent, hind legs may be laid out *a:* when standing extended	*i:* extended or loosely wrapped *a:* tail up or loosely downwards	laid on the surface or over the body, some movement	closed, half opened or normal opened	normal	half back (normal) or erected to front	lateral (normal) or forward (normal)	none	sleeping, resting, alert or active, may be playing
3 Weakly tense	*i:* laid ventrally or sitting *a:* standing or moving, back horizontal	not exposed, normal ventilation	*i:* bent *a:* when standing extended	*i:* on the body or curved backwards, may be twitching *a:* up or tense . downwards, may be twitching	over the body, some movement	normal opened	normal	half back (normal) or erected to front or back and forward on head	lateral (normal) or forward	meow or quiet	resting, awake or actively exploring
4 Very tense	*i:* laid ventral, rolled or sitting *a:* standing or moving, body behind lower than in front	not exposed, normal ventilation	*i:* bent *a:* when standing hind legs bent, in front extended	*i:* close to the body *a:* tense downward or curled forward, may be twitching	over the body or pressed to the body, little or no movement	widely opened or pressed together	normal or partially dilated	erected to front or back, or back and forward on head	lateral (normal), forward or back	meow, plaintive meow or quiet	Cramped sleeping, resting or alert, may be actively exploring, trying to escape
5 Fearful, stiff	*i:* laid ventrally or sitting *a:* standing or moving, body behind lower than in front	not exposed, normal or fast ventilation	*i:* bent *a:* bent near to surface	*i:* close to the body *a:* curled forward close to the body	on the plane of the body, less or no movement	widely opened	dilated	partially flattened	lateral (normal), forward or back	plaintive meow, yowling, growling or quiet	alert, may be actively trying to escape
6 Very fearful	*i:* laid ventrally or crouched directly on top of all paws, may be shaking *a:* whole body near to the ground, crawling, may be shaking	not exposed, fast ventilation	*i:* bent *a:* bent near to surface	*i:* close to the body *a:* curled forward close to the body	near to surface, motionless	fully opened	fully dilated	fully flattened	back	plaintive, meow, yowling, growling or quiet	motionless alert or actively prowling
7 Terrorized	*i:* crouched directly on top of all fours, shaking a: not applicable	not exposed, fast ventilation	*i:* bent *a:* not applicable	*i:* close to the body *a:* not applicable	Lower than the body, motionless	fully opened	fully dilated	fully flattened back on head	back	plaintive meow, yowling, growling or quiet	motionless alert

2.2.6 Approach test

An approach test was used to record the cats' responses to humans, derived from McCune (1994). At the start of each approach test, the author approached the cage from the front and greeted the cat with 'hello cat', touched the cage door with one hand and blinked once at the cat. He remained for 1 minute, observing but not interacting with the cat. 'Hello cat' was repeated after 30 seconds. During the minute's observation time the author recorded latencies to certain behaviours, and presence and absence of others (Table 2.3). As with CSS, if a cat was obviously disturbed shortly prior or during the test, he returned to it later to carry out the test.

Table 2.3 Measures recorded during the approach test

Measure	*Definition*
Back start	Whether the cat is at the front or the back half of the cage at the start of the test
Back end	Whether the cat is at the front or the back half of the cage at the end of the test
Time back	Total time the cat spent at the back of the cage during the test
Num crosses	Number of times the cat crossed over the line separating the front and back halves of the cage
Vocalization	How many meows, mews or similar the cat uttered during the test. (Agonistic vocalisations, such as hisses, spitting, growling were not counted as they are included in 'hiss-spit')
Post approach	Latency to perform a postural approach – part of the cat moving towards the observer
Post withdrawal	Latency to perform a postural withdrawal
Body approach	Latency to perform a body approach – the whole body moving towards the observer. Requires the cat to either start walking towards observer, or a major postural shift
Body withdrawal	Latency to perform a body withdrawal
Touch latency	Latency to touch the observer
CSS	Cat Stress Score, assessed throughout test
ASR	Whether the cat was alert, asleep or resting at the start of the test
All the following were recorded as coded measures – presence or absence of the behaviour during the minute. Behaviours are defined as:	
Friendly	A general impression of friendliness as perceived by the observer
Unfriendly	A general impression of unfriendliness as perceived by the observer
Ignore	Cat spends > 5 s looking away from the observer
Observe	Cat looks at observer
Flatten	Flattened posture: If active, whole body is near to ground, elbows highly bent. If inactive, laid ventrally, head low
Hiss--spit	Hiss, spit, growl or stare (continued eye contact with the observer's eyes for > 2 s)
Piloerection	Hair standing up
Sniff	A sniff directed at the observer, when the cat is within 10 cm
Rub	Cat rubs, or attempts to rub, the observer's hand
Purr	Cat purrs
Eat	Eats food
Groom	Grooms self

2.2.7 Data analysis

CC ratio

The urine collected each morning was used to generate the CC ratio for the preceding day, as it contained urine collected in the bladder and voided in the previous 24 hours. As a further validation of this study's assays, subsamples of twelve of the urine samples assayed by ELISA (Section 2.23) were sent to Cambridge Specialist Laboratory Services (Swanston, Cambridge, UK) for analysis by radioimmunoassay (RIA). The Spearman correlation coefficient between the two sets of results was high: 0.860 ($p < 0.01$), though the absolute levels reported by RIA were a factor of two higher than those obtained by the ELISA. The ELISA data will be used throughout this chapter.

Cat Stress Score

The four Cat Stress Scores collected on an observation day were averaged for each cat, as the scores within a day very rarely differed by more than one level (q.v. Kessler & Turner 1997). For days when the cat had less than 4 scores, such as first day of admission, the scores obtained were averaged.

Approach Test

Data reduction

To investigate which of the many variables measured were discriminating, histograms of each variable were generated using data from all cats and all days to examine the distribution of values. All variables were then compared using Spearman rank correlations (leaving out the non-binary categorical variables) for admissions data only. All statistical analyses were conducted on SPSS for Windows release 11.0, SPSS Inc. Although the Spearman rank correlation should not be used on binary data, it is often considered acceptable for exploring data, so presence or absence measures were included (Table 2.4).

Table 2.4 Spearman correlations of raw admissions data

Correlation coefficients shown, * = $p < 0.05$, ** = $p < 0.01$.

Significant +ve correlations shaded pink, sig -ve correlations shaded yellow

Group 1 shaded blue, group 2 shaded purple, group 3 shaded green, group 4 shaded orange

	Backstart	Numcross	Backend	Timeback	Vocal	Postapp	Postwith	Bodyapp	Bodywith	Touch	CSS	Friendly	Unfriendly	Ignore
Backstart	.													
Numcross	.122**	.												
Backend	.650**	-.263**	.											
Timeback	.760**	-.332**	.888**	.										
Vocal	-.139**	.165**	-.221**	-.228**	.									
Postapp	.260**	-.373**	.392**	.462**	-.193**	.								
Postwith	.304**	-.279**	.307**	.375**	-.126**	.681**	.							
Bodyapp	.106*	-.670**	.418**	.479**	-.319**	.577**	.410**	.						
Bodywith	.214*	-.595**	.335**	.446**	-.280**	.495**	.484**	.791**	.					
Touch	.255**	-.466**	.550**	.564**	-.340**	.521**	.434**	.715**	.628**	.				
CSS	.133**	-0.055	.242**	.280**	.007	.234**	.180**	.144**	.094*	.315**	.			
Friendly	-.172**	.513**	-.528**	-.527**	.360**	-.484**	-.369**	-.715**	-.625**	-.924**	-.270**	.		
Unfriendly	-.128**	-.010	.073	.058	.000	-.039	-.109**	.033	-.038	.025	.170**	-.097*	.	
Ignore	.089*	-.125**	.113**	.142**	-.094*	.111**	.048	.198**	.139**	.248**	.133**	-.228*	-.176**	.
Observe	-.124**	.159**	-.175**	-.196**	.137**	-.328**	-.317**	-.221**	-.209**	-.186**	-.050	.180**	.046	-.259**
flattened	-.109**	-.018	.009	-.010	-.048	.035	-.020	-.016	-.074	.033	.215**	-.077	.253**	-.001
Hiss-spit	-.114**	-.056	.036	.020	.080	.032	-.040	.045	.011	-.006	.184**	-.052	.640**	-.156**
piloerect	-.127**	-.022	.032	.036	-.019	-.065	-.130**	.031	-.038	-.032	.093*	-.063	.650**	-.115**
sniff	-.123**	.454**	-.378**	-.435**	.250**	-.444**	-.275**	-.613**	-.519**	-.549**	-.221**	.558**	-.090*	-.214**
rub	-.126**	.523**	-.488**	-.484**	.341**	-.472**	-.352**	-.712**	-.605**	-.906**	-.271**	.953**	-.095*	-.240**
purr	-.153**	.268**	-.353**	-.367**	.189**	-.367**	-.256**	-.376**	-.295**	-.537**	-.376**	.560**	-.119**	-.275**
eat	-.146**	.158**	-.354**	-.310**	.157**	-.275**	-.229**	-.334**	-.329**	-.407**	-.138**	.403**	-.047	.161**
groom	-.112**	-.046	-.052	-.049	-0.023	-.092*	-.112**	.019	-.048	.022	-.019	-.031	-.040	.084*
hide	-.063	-.079	.088*	.099*	-0.039	.121**	.083*	.064	.017	.092*	.240**	-.090*	-.022	.128**

Table 2.4 Spearman correlations of raw admissions data

Correlation coefficients shown, * = $p < 0.05$, ** = $p < 0.01$.

Significant +ve correlations shaded pink, sig -ve correlations shaded yellow

Group 1 shaded blue, group 2 shaded purple, group 3 shaded green, group 4 shaded orange

	Observe	Flattened	Hiss-spit	Piloerec	Sniff	Rub	Purr	Eat	Groom	Hide
Backstart										
Numcross										
Backend										
Timeback										
Vocal										
Postapp										
Postwith										
Bodyapp										
Bodywith										
Touch										
CSS										
Friendly										
Unfriendly										
Ignore										
Observe	.									
flattened	-.015	.								
Hiss-spit	.016	.150**	.							
piloerect	.030	.401**	.416**	.						
sniff	.169**	-.072	-.073	-.059	.					
rub	.178**	-.076	-.065	-.062	.569**	.				
purr	.213**	-.113**	-.058	-.092*	.325**	.553**	.			
eat	.090	-.042	-.071	-.030	.158**	.358**	.239**	.		
groom	.048	-.032	-.032	-.026	.009	-.027	-.003	-.057	.	
hide	-.275**	.276**	-.035	-.015	-.084*	-.088*	-.107**	-.050	-.037	.

The measures appeared to fall into four main groups in which each measure was significantly correlated with most of the other measures in the group, but was uncorrelated or negatively correlated with measures outside the group (Table 2.5).

Table 2.5 Groups of approach test data derived from Spearman rank correlations of raw admissions data. For definitions, see Table 2.3.

Group 1	backstart, backend, timeback, postapp, postwith, bodyapp, bodywith, touch, CSS, ignore, hide
Group 2	numcross, vocal, friendly, observe, sniff, rub, purr, eat
Group 3	unfriendly, flatten, hiss-spit, piloerect
Group 4	groom

Measures in each group were uncorrelated with measures in other groups, apart from measures in group1, which were significantly negatively correlated with most measures in group 2, and *vice versa*. Group 1 was interpreted as being measures associated with being in the back of the cage and not moving / avoiding being noticed, a passive coping strategy; group 2 was interpreted as friendly behaviours and /or involving movement and being noticed; group 3 as unfriendly or warning behaviours; group 4 was grooming, which was unassociated with anything else.

Cluster analysis was then used to produce a more accurate definition of natural groups. No cluster analysis method was available which could be used with a mix of continuous and binary data. From visual inspection of the histograms, reducing most of the continuous variables to binary data did not cause the loss of much information. Continuous / categorical data was converted to binary data as described in Table 2.6. This binary data was subjected to Spearman rank correlations, and gave very similar groupings as the raw data, confirming that little information had been lost.

Table 2.6 Approach test data converted to binary form for cluster analysis

Original measure	Conversion to binary
Backstart	1 = Cat started test at back half of the cage, 0 = at front
Backend	1 = Cat ended test at back half of cage, 0 = at front
Timeback	1 = Cat spent ≥ 30 secs in back half of cage, 0 = < 30s
Numcross	1 = Cat switched halves of the cage at least once, 0 = no switching
Postapp	1 = Cat made a postural approach during the test, 0 = did not
Postwith	1 = Cat made a postural withdrawal during the test, 0 = did not
Bodyapp	1 = Cat made a body approach during the test, 0 = did not
Bodywith	1 = Cat made a body withdrawal during the test, 0 = did not
Touch	1 = Cat touched observer during the test, 0 = did not
ASR	1 = Cat was alert at start of test, 0 = cat was resting or asleep at start of test

Cluster analysis was then performed on the binary data. Admissions block data and cattery data were analysed separately due to the large differences in housing. Data from catteries 1 and 2 were combined as 'cattery data' as the housing was similar.

Using the binary squared Euclidean distance matrix and the average linkage clustering method gave the dendrograms shown in Figures 2.5a and b. A cut off distance of 9 was decided on as it resulted in groups which seemed to make biological sense. Groupings from the two analyses are summarized in Table 2.7.

Figure 2.5a Hierarchical cluster analysis for admissions approach test data.
Dendrogram using average linkage between groups based on binary squared Euclidean distance matrix. Based on 574 data from 25 cats. Taking the cut-off Distance as 9, groupings are separated by double lines.

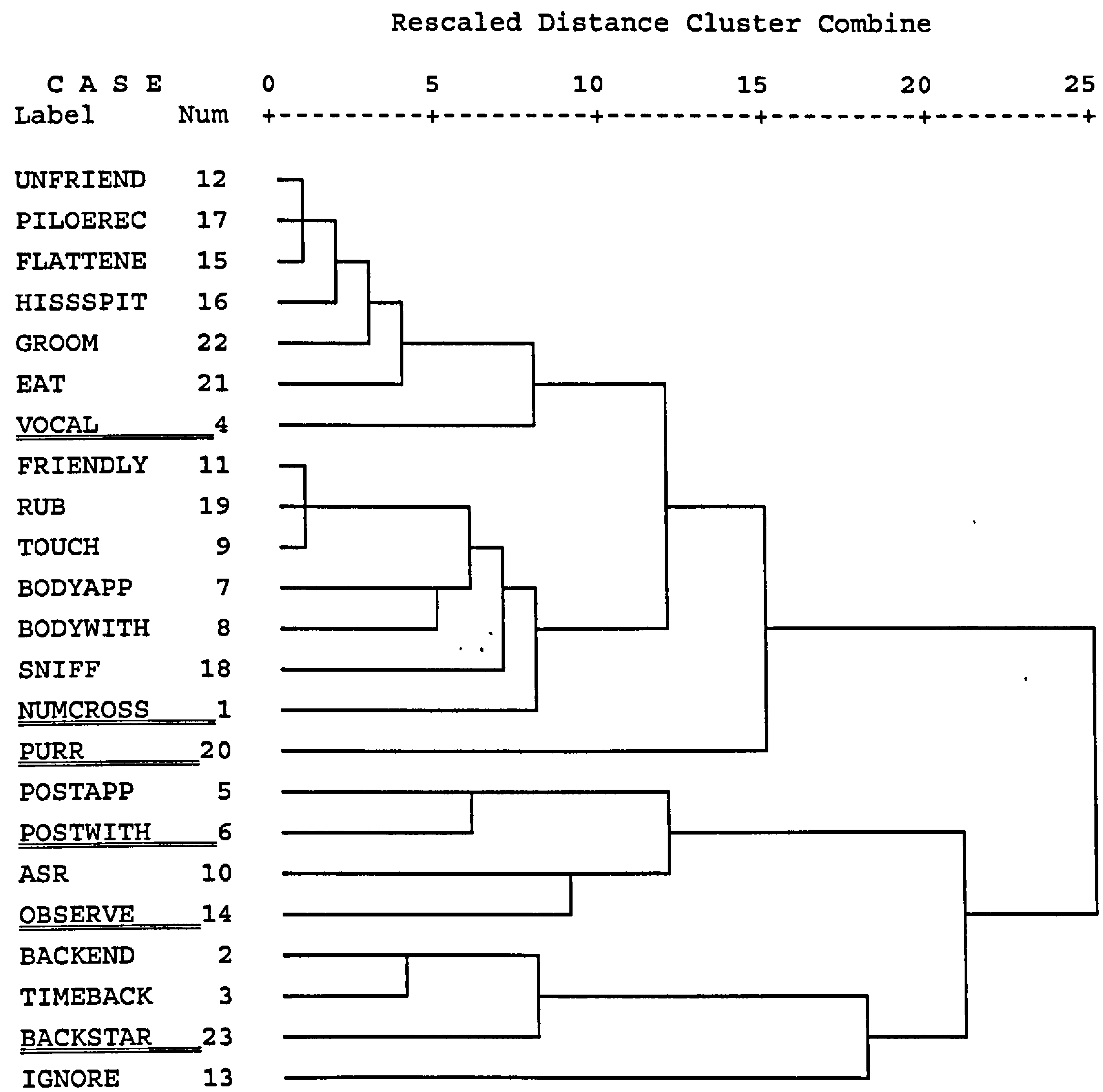


Figure 2.5b Hierarchical Cluster analysis for cattery approach test data.
Dendrogram using average linkage between groups based on binary squared Euclidean distance matrix. Based on 357 data from 17 cats. Taking the cut-off distance as 9, groupings are separated by double lines.

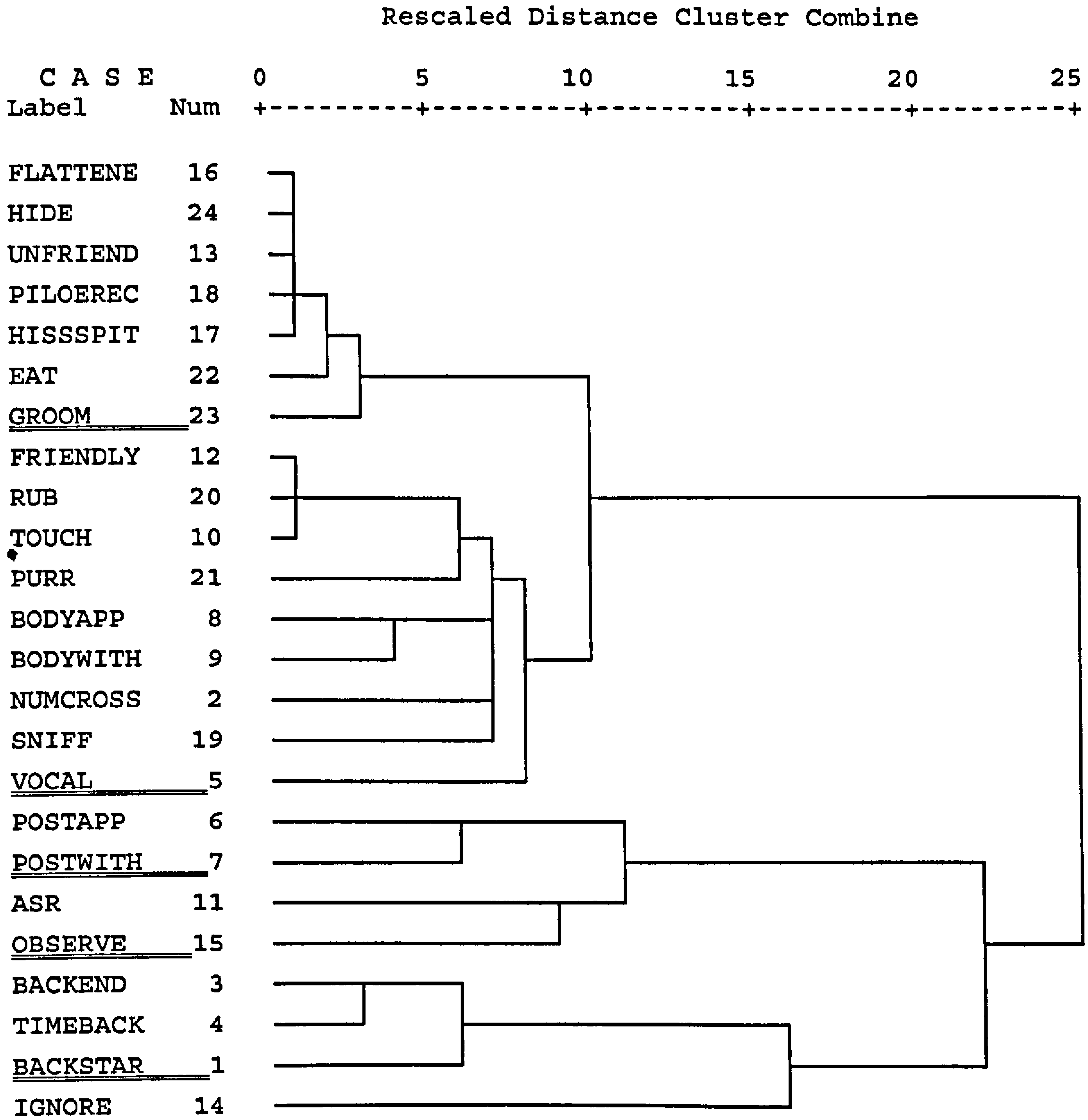


Table 2.7 Groupings of approach test measures as defined by the cluster analyses (Figure 2.5).

Admissions:
Group 1: Unfriendly, Piloerect, Flattened, Hide, Hiss-spit, Groom, Eat, *Vocal*
Group 2: Friendly, Rub, Touch, Bodyapp, Bodywith, Sniff, Numcross
Group 3: *Purr*
Group 4: Postapp, Postwith
Group 5: Asr, Observe
Group 6: Backend, Timeback, Backstart
Group 7: Ignore

Cattery:
Group 1: Unfriendly, Piloerect, Flattened, Hide, Hiss-spit, Groom, Eat
Group 2: Friendly, Rub, Touch, Bodyapp, Bodywith, Sniff, Numcross, *Vocal, Purr*
Group 3: Postapp, Postwith
Group 4: Asr, Observe
Group 5: Backend, Timeback, Backstart
Group 6: Ignore

In admissions, 'vocal' is grouped with group 1 (unfriendly / fearful / displacement behaviours) although it is the most distant from all other measures in the group, but in the cattery dataset it groups with group 2 (friendly / affiliative behaviours) and is again the most distant from all other measures in the group. This suggests that some cats may have used vocalizations as an indicator of fear and others as an affiliative signal, and that the relative proportions of these cats changed. This may have been due to increasing time spent at the shelter (and thus increasing adjustment), or to some property of the cattery housing resulting in increased confidence, such as being able to escape to the outside room.

'Purr' changes from being distantly related to groups 1 and 2 in the admissions block to being part of group 2 (friendly / affiliative behaviours) in the cattery block. This may be due to similar changes in motivation as occurred for vocalizations, and emphasizes that purring is not simply a signal of happiness as many cat owners assume. "Purring may be a manipulative contact- and care- eliciting signal" (Bradshaw *et al.* in prep).

Due to these changes in the grouping of 'vocal' and 'purr', they were put into groupings of their own. The final groupings for analysis are in Table 2.8. Group 1 was interpreted as including fearful / displacement behaviours, and will henceforth be referred to as *fearful*. Group 2 was interpreted as containing friendly / affiliative behaviours, henceforth *affiliative.* Group 3 contained postural changes, henceforth *postural.* Group 4 was interpreted as containing alert, interested-in-surroundings behaviours, henceforth *alert.* Group 5 contained three different measures of being at the back of the cage, henceforth *back.* Groups 6, 7, 8 each contain a single behaviour pattern; *ignore, purr* and *vocal* respectively.

Table 2.8 Final groupings of approach test measures

Group 1: 'Fearful': Unfriendly, Piloerect, Flattened, Hide, Hiss-spit, Groom, Eat
Group 2: 'Affiliative': Friendly, Rub, Touch, Bodyapp, Bodywith, Sniff, Numcross
Group 3: 'Postural': Postapp, Postwith
Group 4: 'Alert': Asr, Observe
Group 5: 'Back': Backend, Timeback, Backstart
Group 6: Ignore
Group 7: Purr
Group 8: Vocal

'Groom' and 'Eat' are included in Group 1, even though they were grouped differently by correlation (Table 2.5), due to their short distance from 'Unfriendly', 'Piloerect', 'Flattened', 'Hide' and 'Hiss-spit'. Grooming is frequently a displacement activity, indicating anxiety. 'Eat', though it may be a displacement behaviour, required the cat to come to the front of the cage right beside the observer. Since 'eat' required the cat to have food left since its last feed approach test (at least an hour after feeding), this suggests that the cat was too fearful to eat the food earlier and did so during the approach test due to social facilitation.

Of these groupings, *fearful* and *affiliative* contain behaviours which are not directly linked to fear or friendliness. *Fearful* includes both active (such as 'hiss-spit') and passive (eg 'hide') defensive behaviours. As well as affiliative behaviours, *affiliative* contains movement behaviours 'bodyapp', 'bodywith' and 'numcross'. This is most likely due to most cats starting off the test at the back of the cage, so any friendly cats had to move to the front of the cage to meet the observer. Fearful cats were generally towards the back of the cage, so had no need to move. Movement is therefore associated with friendliness in the majority of approach tests.

A new variable was coded for each group, being the average of all (binary) measures in it. The two approach tests for each day were then averaged to give one datum per group per cat per day. All further statistics carried out on approach test data used these eight variables (*fearful, affiliative, postural, alert, back, ignore, purr* and *vocal*).

Changes over time

To look at how each variable (CC, CSS and the 8 approach test variables) changed as the cat adapted to its surroundings, each variable for each cat was plotted against time. The slopes of the curve were lines of best fit. The slopes were then used to examine temporal changes rather than using actual values, as this averaged out errors in recording and reduced day-to-day variations in temperament, as well as reducing the number of statistical tests required.

By visual inspection of how many days a cat was present for (Figures. 2.6a, 2.6b) it was decided to generate curves over the first 8 days in the admissions block, and the first 7 days in the cattery block.

Figure 2.6a Length of time cats were present in the admissions block (days since introduction).

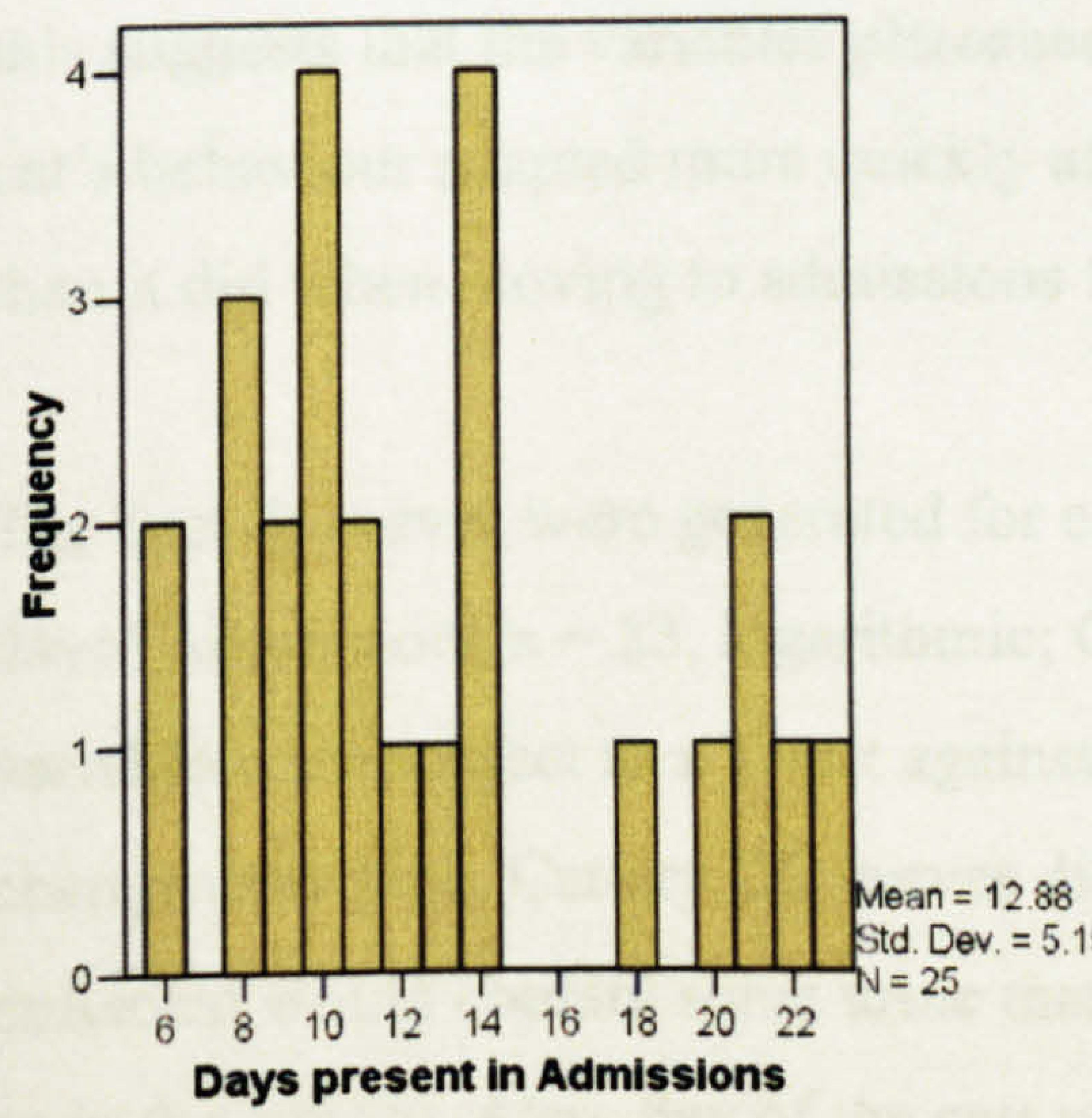
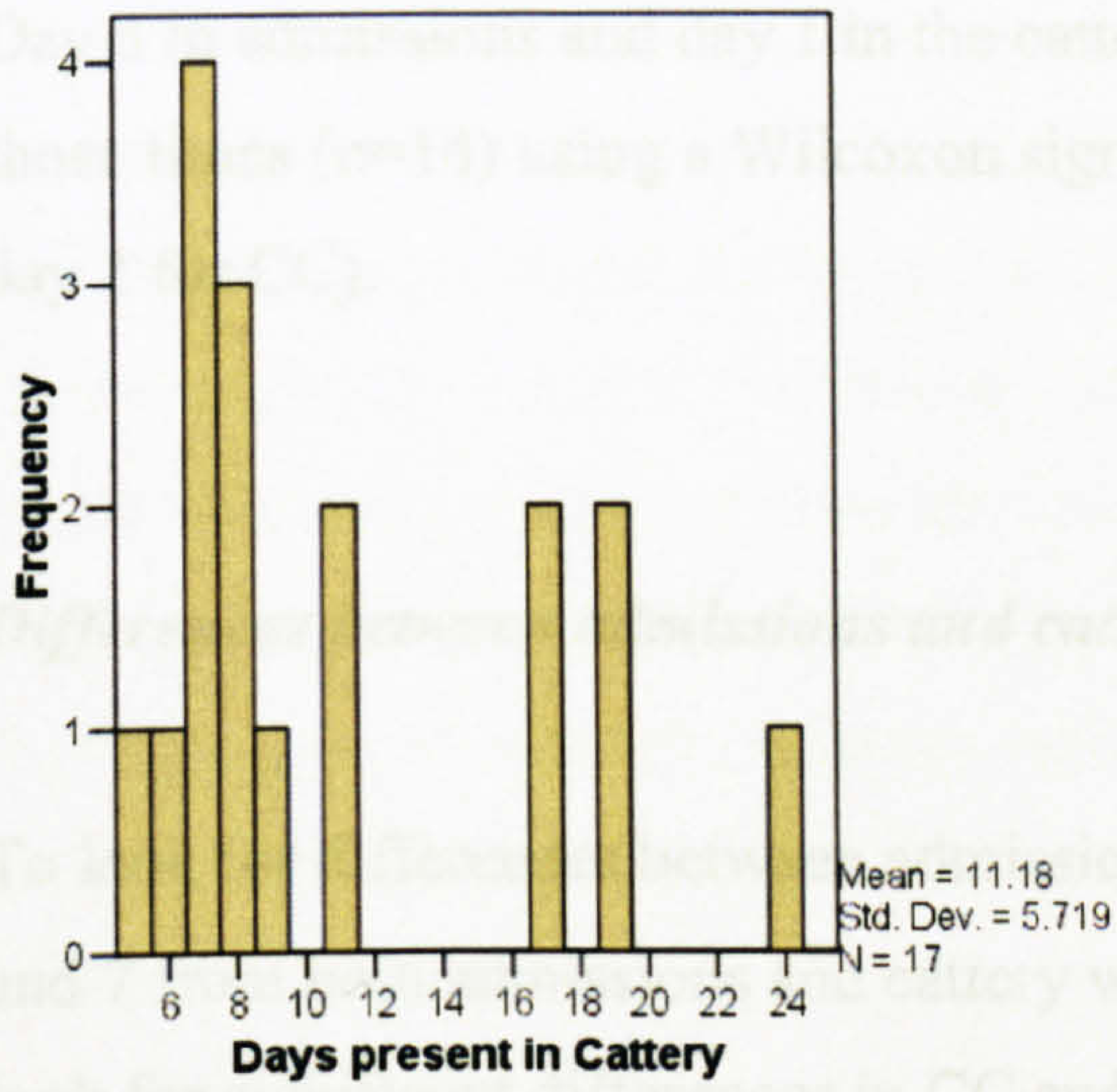


Figure 2.6b **Length of time cats were present in the cattery block (days since move from admissions)**



Using the average data for all cats present in admissions for 8 days or more, curves were generated for each variable. The curves generated were linear, logarithmic, inverse, power, compound, growth and exponential; quadratic and cubic were not used due to unrealistic projections after 8 days. R-squared values were compared, the best overall fit being logarithmic [$y = b0 + (b1.\ln(t))$], where b0, b1 and t are the estimated day 1 value, slope of the line, and time, respectively. The same was carried out on cattery data for cats present for 7 days or more: the best curve was inverse [$y = b0 + (b1/t)$]. Since inverse curves plateau off more quickly than logarithmic ones, this suggests that the variables plateaued off more quickly in the cattery, and that the cat's behaviour adapted more quickly after moving to the cattery from admissions than it did when moving to admissions from a home environment.

The best fit curves were generated for each cat present for the requisite number of days (Admissions n = 23, logarithmic; Cattery n = 15, inverse). The slopes of each variable were subject to a T-test against a mean of zero to check for a significant change over time. Cattery CC curves did not include day 1 values, since the first urine collected would contain some urine that collected in the bladder in admissions as well as in the cattery. Also, few of the cats urinated on day 1 (which may be a sign of stress in itself), but nearly all did on day 2. If the less stressed cats were the ones who urinated on day 1, this would bias that day's data downwards. Because of this, the day 1 cattery CC ratios were excluded from further analysis.

Changes upon transfer to cattery block

Day 8 in admissions and day 1 in the cattery were compared for all cats present at those times (n=14) using a Wilcoxon signed ranks test for each variable (day 8 and day 2 for CC).

Differences between admissions and cattery

To look for differences between admissions and cattery data, mean data from days 6 and 7 from both admissions and cattery were used. Paired sample T-tests were used to look for significant differences in CC and CSS.

Correlations between variables (individual level)

At the level of the whole population being studied, CSS, CC and some of the approach test variables appeared to follow similar or mirror-image time-courses (Figures. 2.7 and 2.8, in sections 2.3.1 and 2.3.2). Superficially, it might be concluded that these are equivalent and interchangeable measures of welfare, all of which reflect the reduction in stress as the cats acclimatise to the shelter conditions. However, these time-courses could also result from two other causes: (a) changes in behaviour which coincide in time with changes in stress but are separately motivated, e.g. behaviour patterns which the cats learn are useful in getting or avoiding the attention of shelter staff, and (b) alternative strategies for coping with stress, i.e. some cats showing stress in one way, other cats in another. For two measures of welfare to be truly equivalent, they should track one another within individuals.

To test for this, correlation coefficients between measured variables within individuals were calculated, and tested for consistency by comparison with a mean of zero. This tests whether measures covary within individuals, irrespective of how long they had been in the shelter and of whether values change consistently over time.

Correlation coefficients between variables were taken for each cat, using data from all days the cat was present in the admissions block. Coefficients for each variable from all cats were tested with a T-test against zero to look for a change over time. Correlations between all variables were not carried out to reduce multiplicity, so only correlations between the variables of most interest were chosen. CC and CSS were picked as they both claim to measure underlying stress, rather than a particular behaviour, and would be of most use in a shelter or research setting if validated. The same analysis was carried out for cattery data (excluding day 1 CC) and 'all' data (both admissions and cattery data considered together). Correlations between CSS and *fearful* were not considered due to the overlap between *fearful* and CSS scores of 4 or higher.

Correlations between intercepts and slopes between cats (population level)

To further evaluate which of the variables measured reflect an underlying reduction in stress as the cat adapts to its new surroundings, correlations between the slopes and intercept (estimated day 1 values) were carried out across individuals. As above, only correlations with CC and CSS were considered to reduce multiplicity. Spearman non-parametric correlations were carried out between admissions CC intercept and slope values and all other admissions intercepts and slopes, and between CSS intercept and slope values and all other intercepts and slopes. The same was carried out for cattery data (excluding day 1 CC values). Although correlations between intercepts and slopes were carried out to help elucidate which of the correlations were causal, they were not themselves discussed as drawing clear conclusions from them is very difficult.

Day 1 values indicate absolute levels of each measure at (presumably) the most stressful point, i.e. whether a cat which responds to stress by performing measure A also responds to stress by performing measure B (positive correlation) or inhibition of measure C (negative correlation). Correlations between slopes indicate whether the overall time-courses for a pair of measures are similar.

Since a day or two of missing CC data was quite common (cats occasionally went an entire day without urinating), two sets of curves were generated for each cat – one only using days for which a complete set of all variables was present for, the other using days for which a complete set of CSS and the 8 approach test variables (i.e. all except CC) were present for. The latter often contained one or two more days' data than the former, allowing a more accurate curve estimate. All Spearman correlations with CC intercept and slope were carried out on the former set of curves, all correlations with CSS intercept and slope carried out with the latter, to maximise the data points in these curves.

2.3 Results

2.3.1 Changes over time following admission – all cats

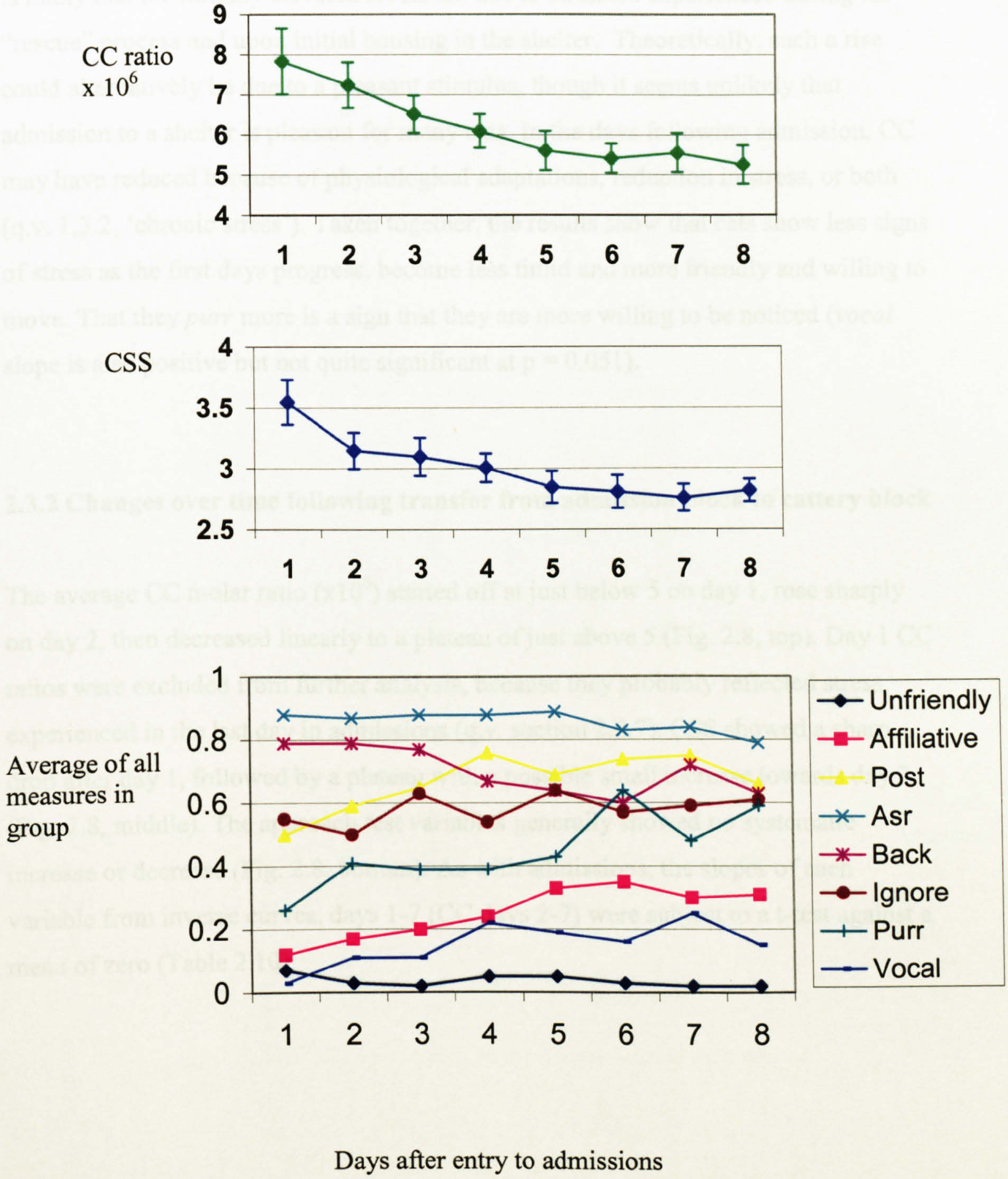
The average CC ratio started at just below 8 (mol cortisol:mol creatinine x10^6) on day 1 and then declined more or less linearly to a little over 5 (Fig. 2.7, top), while CSS had a sharp drop between days 1 and 2, followed by a gentler decline to a plateau of between 2.75 and 3, a little below 'weakly tense' (Fig. 2.7, middle). The approach test variables have less obvious trends, though many variables do seem to change qualitatively over time (Fig 2.7, bottom). This was formally tested by subjecting the slopes of each variable calculated for each individual, to a T-test against a mean of zero (Table 2.9). This test of slopes showed that CC, CSS and *back* had significant negative slopes, and *affiliative, postural* and *purr* had significant positive slopes. *Fear* and *alert* had non-significant negative slopes, and *ignore* and *vocal* non-significant positive slopes.

Table 2.9 T-test of slopes against a mean of zero (2-tailed) from curves generated individually for each cat (n=23). Slope is b1 in the equation for the logarithmic curve, y=b0+(b1.ln(day)), i.e. a positive slope indicates an increasing value of y over time (though the amount of increase declines with increasing day).
** Difference is significant at the .01 level (2-tailed).
* Difference is significant at the .05 level (2-tailed).
Significant +ve t-values shaded in purple, -ve in yellow.

Admissions, n=23

Var	Mean value	T	Sig. (2-tailed)
CC SLO	-1.544	-4.37**	0.000
CSS SLO	-0.342	-6.77**	0.000
FEAR SLO	-0.008	-0.845	0.407
AFF SLO	0.115	3.22**	0.004
POST SLO	0.111	2.47*	0.022
ALERT SLO	-0.036	-1.59	0.124
BACK SLO	-0.096	-2.09*	0.049
IGNO SLO	0.018	0.328	0.746
PURR SLO	0.133	2.66*	0.014
VOC SLO	0.069	2.070	0.051

Figure 2.7 Mean CC, CSS and approach test variables, +/- SE for CC and CSS, plotted against day since entry to the shelter. Admissions only, days 1-8. SE bars where shown. Data from cats staying in admissions for 8 days or more, N=23.



The CC and CSS results show that the cats had a decrease in physiological and behavioural signs of stress over time as they acclimatised to the cattery. Although no measurements could be taken prior to admission, to generate a baseline for each cat, it is likely that the initially elevated levels are due to stressors experienced during the "rescue" process and upon initial housing in the shelter. Theoretically, such a rise could alternatively be due to a pleasant stimulus, though it seems unlikely that admission to a shelter is pleasant for many cats. In the days following admission, CC may have reduced because of physiological adaptations, reduction in stress, or both (q.v. 1.3.2, 'chronic stress'). Taken together, the results show that cats show less signs of stress as the first days progress, become less timid and more friendly and willing to move. That they *purr* more is a sign that they are more willing to be noticed (*vocal* slope is also positive but not quite significant at $p = 0.051$).

2.3.2 Changes over time following transfer from admission block to cattery block

The average CC molar ratio ($x10^6$) started off at just below 5 on day 1, rose sharply on day 2, then decreased linearly to a plateau of just above 5 (Fig. 2.8, top). Day 1 CC ratios were excluded from further analysis, because they probably reflected stress experienced in the last day in admissions (q.v. section 2.2.7). CSS showed a sharp drop after day 1, followed by a plateau with a possible small increase towards day 7 (Fig. 2.8, middle). The approach test variables generally showed no systematic increase or decrease (Fig. 2.8, bottom). As with admissions, the slopes of each variable from inverse curves, days 1-7 (CC days 2-7) were subject to a t-test against a mean of zero (Table 2.10).

Figure 2.8 Mean CC, CSS and approach test variables, +/- SE for CC and CSS, plotted against day since entry to the cattery. Cattery only, days 1-7. Data from cats staying in cattery 7 days or more, N=15; day 1 CC N = 7.

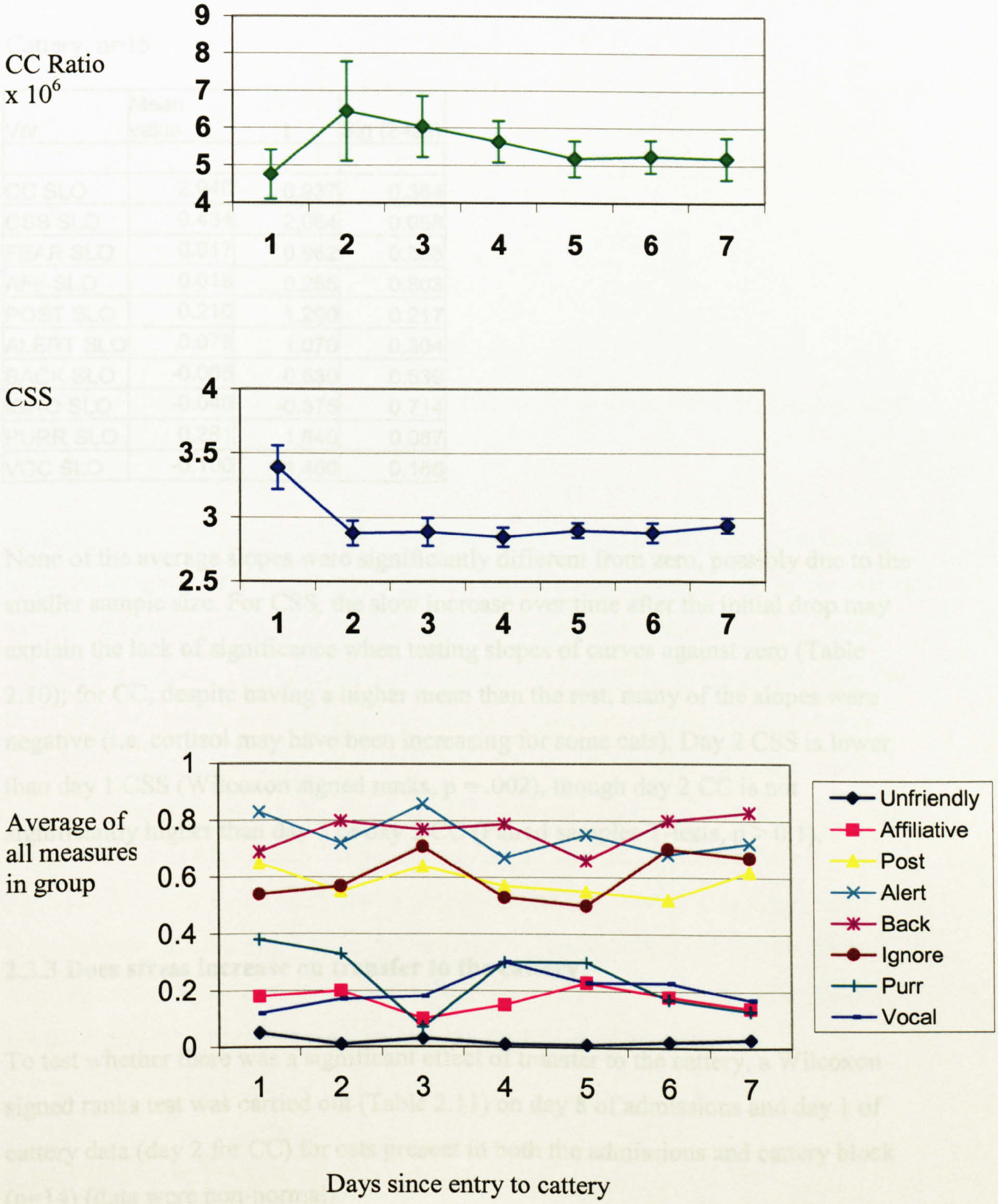


Table 2.10 T-tests of slopes against a mean of zero (2-tailed) from curves generated individually for each cat (n=15). Slope is b1 in the equation for the inverse curve, y=b0+(b1/day), i.e. the estimated drop between day 1 and day ∞, which is positive for values that decline with time. Day 1 for CC omitted (qv *Cattery CC ratios*, section 2.2.7)
** Difference is significant at the .01 level (2-tailed).
* Difference is significant at the .05 level (2-tailed).

Cattery, n=15

Var	Mean value	t	Sig (2-tail)
CC SLO	2.940	0.937	0.364
CSS SLO	0.434	2.064	0.058
FEAR SLO	0.017	0.962	0.353
AFF SLO	0.018	0.255	0.803
POST SLO	0.210	1.290	0.217
ALERT SLO	0.078	1.070	0.304
BACK SLO	-0.065	-0.630	0.539
IGNO SLO	-0.040	-0.375	0.714
PURR SLO	0.281	1.840	0.087
VOC SLO	-0.100	-1.460	0.166

None of the average slopes were significantly different from zero, possibly due to the smaller sample size. For CSS, the slow increase over time after the initial drop may explain the lack of significance when testing slopes of curves against zero (Table 2.10); for CC, despite having a higher mean than the rest, many of the slopes were negative (i.e. cortisol may have been increasing for some cats). Day 2 CSS is lower than day 1 CSS (Wilcoxon signed ranks, $p = .002$), though day 2 CC is not significantly higher than day 3 or day 5 CC (Paired samples T-tests, $p > 0.1$).

2.3.3 Does stress increase on transfer to the cattery?

To test whether there was a significant effect of transfer to the cattery, a Wilcoxon signed ranks test was carried out (Table 2.11) on day 8 of admissions and day 1 of cattery data (day 2 for CC) for cats present in both the admissions and cattery block (n=14) (data were non-normal).

Table 2.11 Wilcoxon signed ranks tests, comparing cattery day1 (day 2 for CC) with admissions day 8. (+) Z-statistic where cattery value higher than admissions, (-)where admissions higher than cattery (overall). Significant Z-values shaded in yellow.

Measure	Z-statistic	p-value
CC	(+)-0.982	0.326
CSS	(+)-2.422	0.015
FEAR	(+)-0.791	0.429
AFF	(-)-0.079	0.937
POST	(+)-1.191	0.234
ASR	(+)-0.765	0.444
BACK	(+)-0.358	0.721
IGNORE	0	1
PURR	(-)-1.387	0.165
VOCAL	0	1

The only variable which was significantly different was CSS, which was higher after transfer to the cattery than day 8 of admissions.

That CSS levels jumped from a plateau of around 2.8 in admissions to 3.4 upon entry to the cattery, showing that the move was stressful, though possibly less so than initial admission (average CSS of 3.55). The next day, it returned to the same level as the admissions plateau. This suggests a fast adaptation to cattery caging, presumably due to its relative similarity to admissions housing and the increased space and retreat possibilities cattery housing offers. CC showed a slower decrease to plateau after around 5 days, similar to admissions, though the peak cattery CC of 6.5 on day 2 was equal to day 3 in admissions. These results suggest that cats are less stressed overall by the move to the cattery than the initial admission to a shelter. This allays any concerns that the cats might be more disturbed by the second move (as this might indicate continual, continued unpredictability in the future of the cats rather than initial admission being a one-off event) than by the initial admission.

2.3.4 Differences between admissions and cattery

To look at differences between admissions and cattery, data from the plateau period were used - average of days 6 and 7 from both admissions and cattery, N=16.

Paired sample T-tests were used to look for significant differences in CC and CSS (Table 2.12). None were found, though the low N gives the test a low power.

Table 2.12 Mean CC and CSS from days 6 and 7, Admissions and cattery. T-value and significance from paired-sample T-tests. N = 16.

Variable	Mean Admiss	Mean Cattery	T-value	Sig
CC	5.54	5.06	.565	.561
CSS	2.80	2.87	-.641	.548

2.3.5 Correlations between variables (individual level)

Correlation coefficients were calculated for admissions data, cattery data and both datasets combined using every day the cat was present in the shelter until taken off the study. The T-values (one-sample t-test vs zero) of these correlation coefficients are shown in Table 2.13, with a summary of the significant results shown in Table 2.14. To reduce multiplicity, only pairings including CC and CSS were performed. For cattery data only, day 1 CC values were excluded.

Table 2.13 T-values for the pairs of variables shown.
** Mean correlation is significantly different from zero at the .01 level (2-tailed).
* Mean correlation is significantly different from zero at the .05 level (2-tailed).
Significant +ve correlations shaded in purple, -ve correlations in yellow.

Admissions data only, n=25

Variables	Mean coeff	T-value	Sig.
CC CSS	0.198	2.459*	0.022
CC Fear	0.093	1.221	0.237
CC Aff	-0.053	-0.709	0.485
CC Post	-0.06	-0.9858	0.335
CC Alert	0.148	2.097*	0.047
CC Back	0.208	2.602*	0.016
CC Ignore	-0.136	-1.448	0.161
CC Purr	-0.024	-0.3	0.767
CC Voc	-0.016	-0.171	0.867

CSS CC	0.198	2.459*	0.022
CSS Aff	-0.234	-2.782*	0.011
CSS Post	-0.073	-1.117	0.275
CSS Alert	0.091	1.11	0.278
CSS Back	0.153	1.762	0.091
CSS Ignore	0.034	0.415	0.682
CSS Purr	-0.066	-0.887	0.385
CSS Voc	-0.099	-1.254	0.228

Cattery data, n=17

Variables	Mean coeff	T-value	Sig.
CC CSS	0.238	1.825	0.093
CC Fear	-0.156	-1.616	0.134
CC Aff	0.015	0.126	0.901
CC Post	0.077	0.706	0.491
CC Alert	0.042	0.358	0.725
CC Back	-0.148	-1.355	0.197
CC Ignore	0.259	2.693*	0.017
CC Purr	-0.006	-0.077	0.940
CC Voc	-0.014	-0.11	0.914

CSS CC	0.196	1.578	0.14
CSS Aff	0.142	1.829	0.087
CSS Post	0.058	0.726	0.478
CSS Alert	0.131	1.39	0.185
CSS Back	-0.244	-2.934**	0.010
CSS Ignore	0.201	2.260*	0.038
CSS Purr	0.048	0.61	0.551
CSS Voc	-0.059	-0.685	0.505

Admissions and cattery data, n=25

Variables	Mean coeff	T-value	Sig.
CC CSS	.212	3.404**	0.002
CC Fear	.063	1.061	0.300
CC Aff	-.080	-1.407	0.173
CC Post	-.050	-1.25	0.272
CC Alert	.130	2.266*	0.033
CC Back	.105	1.656	0.111
CC Ignore	-.044	-.660	0.515
CC Purr	-.030	-.464	0.647
CC Voc	-.031	-.043	0.966
CSS CC	.212	3.404**	0.002
CSS Aff	-.193	-3.589**	0.002
CSS Post	-.076	-1.605	0.122
CSS Alert	.060	.818	0.421
CSS Back	.143	2.292*	0.031
CSS Ignore	.100	1.579	0.127
CSS Purr	-.095	-1.776	0.089
CSS Voc	-.055	-.991	0.333

These tests were conducted to see whether variables that follow similar time courses and positively correlate at the population level, also do so at the individual level. If not, this suggests that they are not measuring the same internal state and/or have different motivations.

All comparisons between CSS and the approach test variables must be interpreted with caution, as all are based on behavioural observations. *Postural*, *Back*, *Ignore* and *Purr* do not have any effect *per se* on CSS. *Alert* and *Vocal* are part of the CSS, though non-significantly correlated here. None of the measures in *Affiliative* are part of CSS, and observation suggests that cats which are friendly may still be stressed. Some of the measures in *Fearful* are part of the CSS - Piloerect, flattened, hiss-spit and many of the indicators for unfriendly are also included in the higher CSS levels - so correlations between CSS and *fearful* were not considered.

Table 2.14 Spearman rank correlation coefficients (rho) between CC and CSS and approach test variables which were significant in either admissions (adm) or cattery (catt).
** Mean correlation is significantly different from zero at the .01 level (2-tailed).
* Mean correlation is significantly different from zero at the .05 level (2-tailed).
Significant +ve correlations shaded in purple, -ve correlations in yellow.

Measures	Mean rho (adm)	Mean rho (catt)
CC CSS	0.198*	0.238
CC Alert	0.148*	0.042
CC Back	0.208*	-0.148
CC Ignore	-0.136	0.259*
CSS Aff	-0.234*	0.142
CSS Back	0.153	-0.244**
CSS Ignore	0.034	0.201*

Only one of these comparisons showed consistency between admissions and the cattery. CC was positively correlated with CSS in both, although the latter is NS, possibly due to the reduced sample size. The mean correlation coefficients were low (0.198 and 0.238 respectively), as were all of the coefficients in this test, but this will in large part have been due to the large day-to-day variation in CC that was not reflected in CSS, which is far more stable than CC between days. So, on average, as individual cats experienced decreases in CC, their CSS also decreased, and vice versa.

In admissions, CC was positively correlated with *Alert* and negatively (NS) with *Ignore*; in the cattery, the relationship with *Ignore* was reversed, and became significant, and the correlation coefficient for *Alert* dropped to near zero. These correlations seem to suggest that cats may change their strategies with time; initially, cats which are physiologically stressed will show alertness and look at the observer, but later will no longer be especially alert and will spend more time looking away from him. The latter may indicate an 'I'm not staring at you' sign of deliberate non-challenge, which appears to be confirmed by the positive correlation between CSS and *Ignore* in the cattery. This type of alternation is typical of cat-cat agonistic behaviour (Bradshaw 1992). Alternatively, the changes may be due to individual differences in coping strategies – when stressed, some cats may ignore the observer and others may try to use him in an attempt to relieve their stress. The lack of correlation in cattery *Alert* may be because cats can be farther away from the front of the cage (and therefore the door), so feel more secure.

A change in strategy with time is also suggested by the *Back* variable. *Back* was positively correlated with CC and CSS in admissions, but negatively in the cattery. This suggests that cats which were more stressed initially spent more time at the back of the cage, but that subsequently it was the less stressed cats which went to the back. In this case, being at the back more may have indicated a more relaxed cat which is happy in its bed, and being at the back less may have indicated a more agitated cat which is at the front of the cage, soliciting social contact. By the time of entry to the cattery, the cats had been in the shelter for longer, so may have become more accustomed to shelter staff (and the observer). In admissions cages, cats could sit on the blanket and still be quite close to the front of the cage for affiliative purposes, though cats which showed active affiliative behaviours tended to come towards the front of the cage. Cattery cages were far deeper than admissions cages, and offered no soft resting substrate towards the front of the cage, so cats tended to rest at the back of the cage. This explanation is partially supported by the relationship between CSS and *Affiliative* which is negative in admissions but becomes positive though non-significant in the cattery. Cats were initially least likely to display *Affiliative* when they had their highest CSS, though subsequently may have used affiliative behaviour as a coping strategy.

Since many cats appear to have changed strategies between admissions and the cattery, the 'all' dataset will not be considered except where correlation coefficients have the same sign in both admissions and cattery data (Table 2.15). This is true for the positive correlation between CC and CSS, which has a higher coefficient (+0.212) and is significant in the 'all' dataset at the $p > .01$ level. This suggests that the lack of significance in the cattery data ($p = .093$) was simply due to a low N. CC and *Alert* remain significant in the 'all' dataset, though with no real change to either coefficient or p-value from admissions data.

Table 2.15 Spearman rank correlation coefficients (rho) between CC and CSS which were significant in 'all' data and had rho of the same sign in both admissions and cattery
** Mean correlation is significantly different from zero at the .01 level (2-tailed).
* Mean correlation is significantly different from zero at the .05 level (2-tailed).
Significant +ve correlations shaded in purple, -ve correlations in yellow.

Measures	Mean rho
CC CSS	0.212**
CC Alert	0.130*

2.3.6 Correlations between intercepts and slopes between cats (population level)

Correlations between slopes and predicted day 1 values for each measured variable were carried out for all pairings with CC and CSS (Table 2.16). Significant results are summarised in Table 2.17.

Table 2.16a Spearman correlation coefficients for curve intercepts (predicted day 1 values) and slopes. Equation y=b0+b1.ln(day), intercept = b0, slope = b1.
Admissions data only, days 1-8, n=23.
** Correlation is significant at the .01 level (2-tailed).
* Correlation is significant at the .05 level (2-tailed).
Significant +ve correlations shaded in purple, -ve correlations in yellow.

	CCINT	CCSLO	CSSIN	CSSSLO
CCINT	*			
CCSLO	-.846**	*		
CSSINT	-0.327	.458*	*	
CSSSLO	0.376	-.477*	-.787**	*
FEARINT	-0.241	0.201		
FEARSLO	0.093	-0.067		
AFFNT	0.142	-0.365	-0.261	0.117
AFFSLO	-0.284	0.241	-0.076	0.235
POSTINT	-0.098	-0.122	-.463*	0.167
POSTSLO	0.029	0.033	0.282	-0.025
ALERTINT	0.318	-0.407	-0.059	0.236
ALERTSLO	-.544**	.523*	0.042	-0.146
BACKINT	0.015	0.105	0.137	0.157
BACKSLO	0.226	-0.130	0.045	-0.259
IGNORINT	-0.278	.446*	.441*	-0.292
IGNORSLO	0.292	-0.386	-0.238	0.129
PURRINT	.434*	-.461*	-.607**	.505*
PURRSLO	-0.099	0.081	0.028	-0.017
VOCINT	0.183	-0.345	-0.141	0.086
VOCSLO	-0.239	0.401	0.192	-0.233

Table 2.16b Spearman correlation coefficients for curve intercepts (predicted day 1 values) and slopes. Equation y=b0+b1/day, intercept = b0+b1, slope = b1. Cattery data only, days 1-7 (CC 2-7), n=15. Key as above.

	CCINT	CCSLO	CSSINT	CSSSLO
CCINT	*			
CCSLO	-.836**	*		
CSSINT	0.106	0.126	*	
CSSSLO	0.164	-0.349	-.589*	*
FEARINT	-0.424	0.298		
FEARSLO	.644**	-.528*		
FRIENINT	-0.425	0.286	-0.336	0.000
FRIENSLO	0.229	-0.296	0.098	0.070
POSTINT	0.039	-0.104	-0.173	0.123
POSTSLO	0.089	-0.096	-0.123	0.080
ASRINT	-0.279	-0.039	-0.064	-0.054
ASRSLO	.525*	-0.479	-0.166	0.424
BACKINT	0.458	-0.402	0.055	0.072
BACKSLO	-0.268	0.349	0.325	-0.019
IGNORINT	0.029	0.164	0.202	-0.059
IGNORSLO	-0.354	0.311	0.179	0.007
PURRINT	-0.372	0.331	-0.245	-0.013
PURRSLO	0.458	-.556*	-0.265	0.225
VOCAINT	-0.133	0.007	-0.244	0.062
VOCASLO	0.072	-0.115	-0.259	-0.214

In the data, a variable's intercept and slope are frequently correlated, probably due to floor and ceiling effects. This correlation means that intercept and slope are not independent and may lead to problems with interpreting data. Partial correlations were used to remove relationships that resulted solely from interdependence, see Appendix One. This eliminates many of the significant correlations in Table 2.16. Negative correlations between a variable's intercept and its own slope are biologically uninformative and were not considered. Further, correlations between intercepts and slopes are very difficult to interpret and were also not considered. Removing all such correlations yields the remaining significant correlations (Table 2.17).

Table 2.17 Spearman correlation coefficients for curve intercept (predicted day 1 values) and slopes. Significant correlations (after checking for artefacts by partial correlation and removing correlations between a variable and its own slope) only shown.
** Correlation is significant at the .01 level (2-tailed)
* Correlation is significant at the .05 level (2-tailed)
Significant +ve correlations shaded in purple, -ve correlations in yellow

a) Admissions, n=23

CC Slo	CSS Slo	-.477*
CSS Int	Post Int	-.463*
CSS Int	Ignore Int	.441*
CSS Int	Purr Int	-.607**

b) Cattery, n=15

CC Int	Fear Int	.644**
CC Slo	Purr Slo	-.556*

Considering admissions data first, CSS slope is negatively correlated with CC slope – cats with fast decreases in CC have slower decreases in CSS and vice versa (Figure 2.9). This may indicate differing coping strategies at either end of a cline, with some cats showing little change in behavioural response but a fast decrease in CC, and others the opposite. There is a non-significant negative correlation between CC int and CSS int also (Figure 2.10), which may stem from the same variation in coping strategies.

Figure 2.9 Plot of CC Slope against CSS slope, data from Table 2.16a (admissions curves).

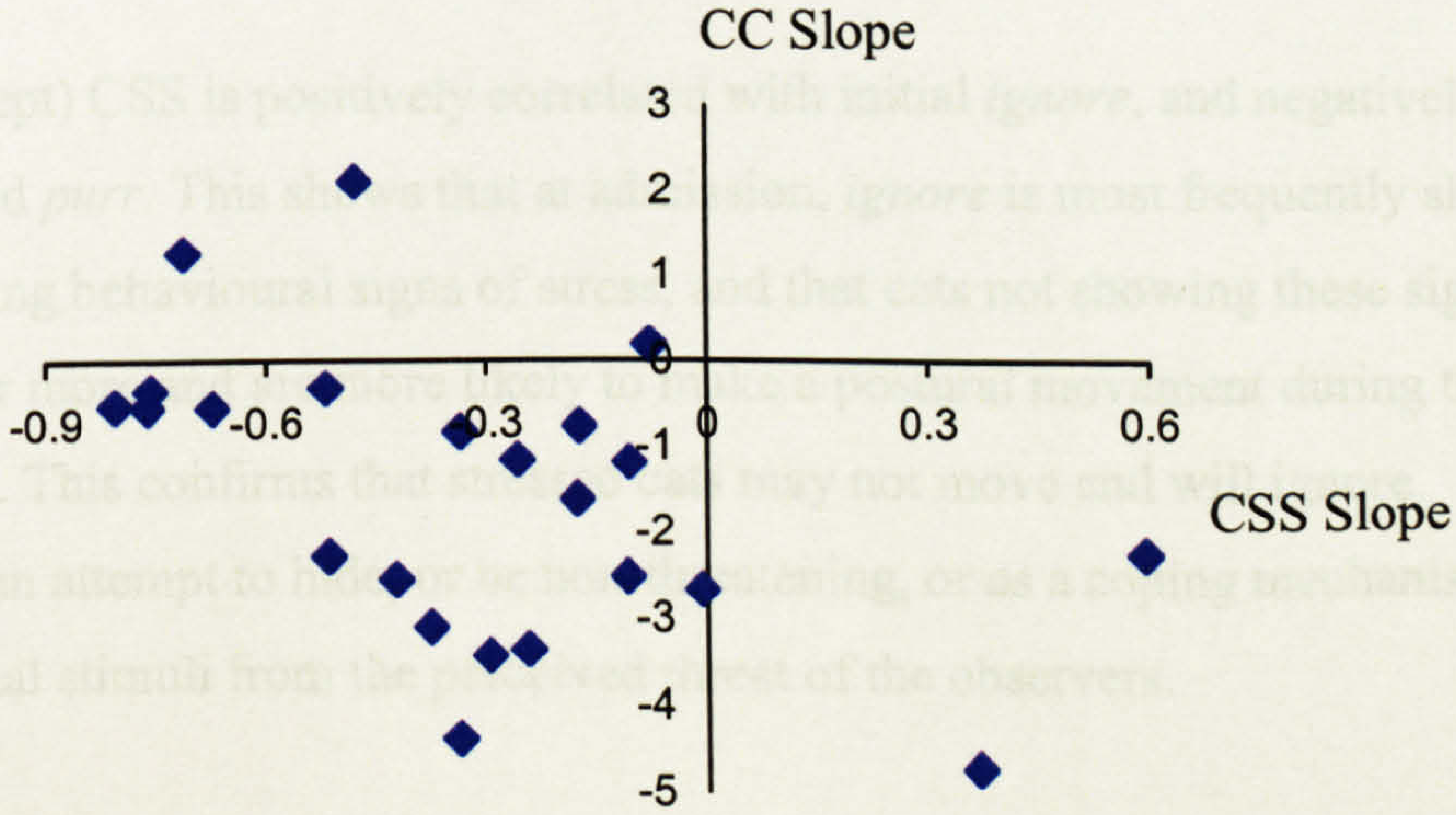
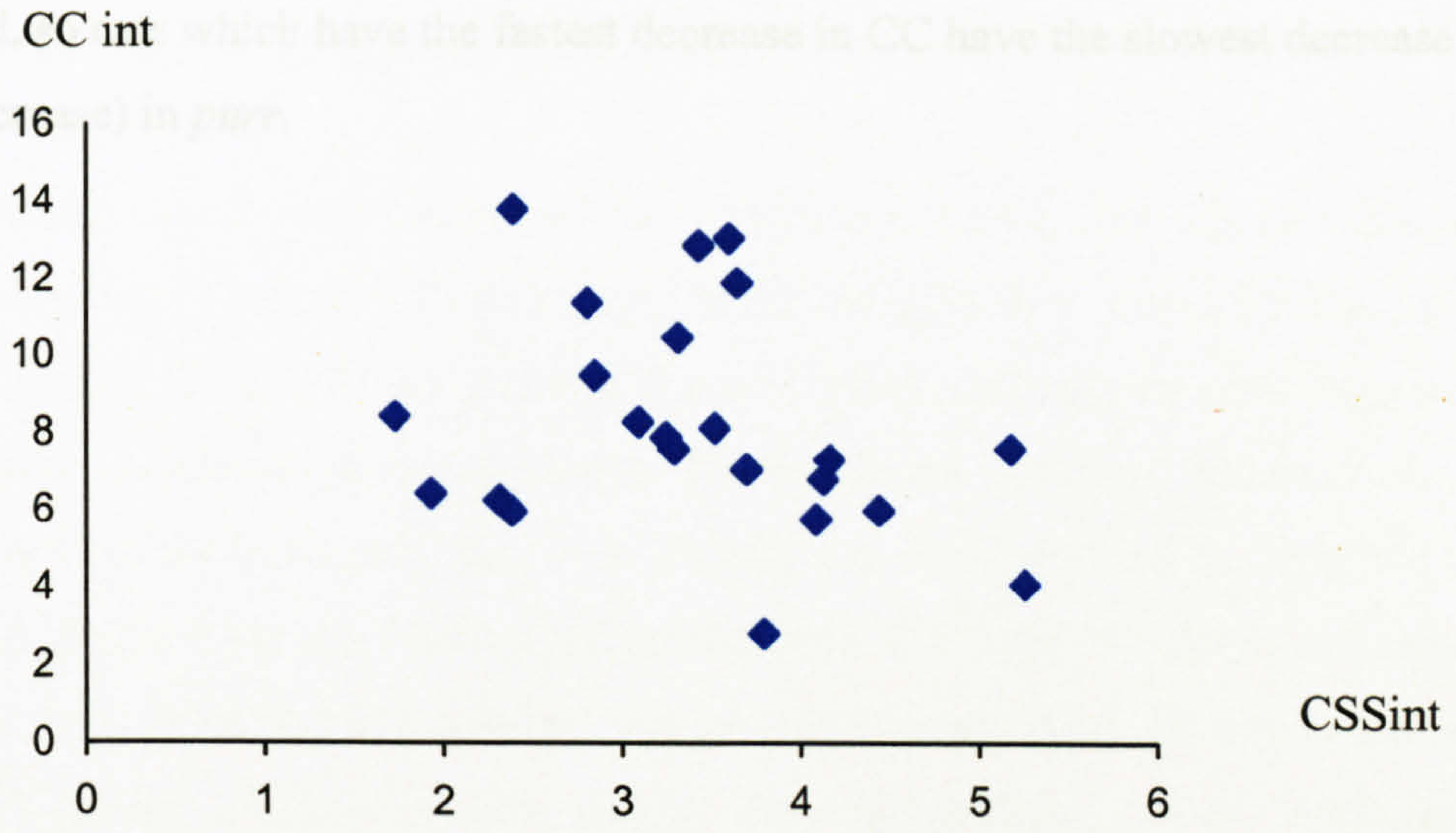


Figure 2.10 Plot of CC intercept against CSS intercept. Data from Table 2.16a (admissions curves)



The correlations between raw variables (Table 2.13) however, are positive. This difference between the two statistical tests may be due to the data reduction in the curve correlations: the between variables correlations are performed on actual data, so the coefficient for each pair of variables per cat takes into account day-to day variation. In contrast, the curve correlations were performed on a summary statistic for each variable. The slopes will 'smooth' out some of the day-to-day variations and summarise rate of change. Although CC and CSS are positively correlated within each cat day by day, between cats, those with fast decreases in CSS tend to have slower decreases in CC, and vice versa.

Initial (intercept) CSS is positively correlated with initial *ignore*, and negatively with initial *post* and *purr*. This shows that at admission, *ignore* is most frequently shown by cats showing behavioural signs of stress, and that cats not showing these signs generally purr more and are more likely to make a postural movement during the approach test. This confirms that stressed cats may not move and will ignore observers in an attempt to hide, or be non-threatening, or as a coping mechanism of avoiding visual stimuli from the perceived threat of the observers.

Turning to the cattery data, CC int is positively correlated with *fear* int – cats with initially high physiological stress as measured by CC have high initial levels of *fear*.

This shows that at least some cats react to stress by showing *fearful* behaviours which would tend to reduce shelter staff contact. CC slope and *purr* slope are negatively correlated, so cats which have the fastest decrease in CC have the slowest decrease (or fastest increase) in *purr*.

2.4 Discussion

2.4.1 Interpreting the groupings of measures

Previous authors have identified behavioural styles in domestic cats. This study's two largest groupings of measures (Table 2.8), *fearful* and *affiliative*, may be equatable to the *bold/shy* trait identified by Meier and Turner (1985), although the groupings were derived from a combination of between-cat and within-cat variation. Feaver *et al.* (1986) identified the traits *sociable / alert / equable to cats*, of which the first two may correspond to this study's affiliative and alert groups. Similarly, the *confident*, *timid* and *active* traits identified in Karsh and Turner (1988) might relate to combinations of this study's groups: *affiliative / vocal*, *fearful / back* and *postural / vocal / affiliative* groups.

Sociable to humans and *generally active* were the most important components extracted by Bradshaw and Cook (1996), but they are unseparated by this study as both were conflated into the *affiliative* group. Although *postural* indicates some activity, this is only in the context of the approach test so cannot be said to be a general measure. This conflation may be because Bradshaw and Cook studied cats in domestic environments where sociability and activity could be separately expressed. In the shelter, there is little opportunity for active behaviours other than the eliciting of human contact, pacing or escape behaviour. Similarly, McCune's (1992) separation of friendliness into *sociability to humans* and *boldness in a novel situation* (largely caused by socialisation and genetic influences, respectively) was not achieved in this study.

2.4.2 Changes over time following admission and transfer to cattery

Admissions: CC, CSS and *back* declined while performance of *affiliative*, *post* and *purr* increased. *Fear* and *alert* had non-significant decreases, and *ignore* and *vocal* non-significant increases. These changes over time will tend to make cats more attractive to potential adopters (Vandenbussche 2001). A possible reason why *fear* and *ignore* do not have significant slopes is that many cats perform these behaviours rarely, which does not give much basis for significant change.

CC and CSS both plateau on day 6, so most cats appear to have overcome their acute stress by then. The plateau of CSS at just below 3 suggests that most cats at this stage are a little below 'weakly tense'. 'Weakly tense' is considered acceptable as a temporary situation by Kessler and Turner (1997), while Ottway and Hawkins (2003) consider 'weakly tense' not to indicate diminished welfare at all.

Cattery: The cats seemed to adapt quicker to the cattery than they did to admissions. CSS returned to baseline by day 2, though CC remained elevated until day 5 (see section 2.44).

2.4.3 Comparisons with previous authors

Comparison of CC ratios with previous authors is hindered as all previous studies known to the author have used radioimmunoassay techniques to measure cortisol, not ELISA as conducted in this study. Carlstead *et al.* (1993) found CC ratios of 1.56 (after conversion from ngCo:mgCr to molCo:Crx10^6 as used in this chapter) in laboratory cats during a baseline period. This period consisted of the normal caretaking regime which the cats were used to, which rose during an experimentally stressful procedure (unpredictable handling and husbandry routine) to a maximum of 2.50. A previous study by the author (Carlstead *et al.* 1992) found a similar baseline CC in laboratory cats. These results, though far lower than those this study, approximate this study's ratio of day 8 admissions (plateau, similar to baseline) to day 1 admissions (5.26 : 7.84). Goossens *et al* (1995) found a median CC in healthy domestic cats in domestic environments of 13.

These large discrepancies between authors using a similar technique are probably due to different methods of analysis in different laboratories – as shown by the twofold difference in samples sent by the author to be analysed by a different laboratory (q.v. section 2.2.8). Rochlitz *et al.* (1997) found a mean ratio of 5.02 for cats in the home environment, and a mean CC on day one of admission to an animal shelter of 9.54.

Rochlitz *et al.* (1998) report a CC ratio on day one of admission to a quarantine cattery of 11.4, with values dropping (non-significantly) to around 6 on days 2 and 3, followed by a plateau of around 5.5 by the second week, decreasing to around 5 by the fifth week. These values (peaks and plateaux) are similar to this study's. In conclusion, other studies have similar drops from day of admission to plateau as this one. McCobb *et al.* (2005) reported a mean CC of 6.8 for all days tested (most data were within the first 20 days) and found almost no correlation with time, though there was a very slight drop. Their mean value is similar to that found by this study, although this study found a significant drop – the author feels that the increased sensitivity in this study is due to repeated measures on each cat (McCobb *et al.* was cross-sectional, taking one datum from each cat, covering a range of days), and restricting observations to just one shelter, rather than 4 in their study. Further, since CC appears to plateau after 6 days or so, the large number of cats which McCobb studied after day 6 (the mean time spent at the shelter was 13 days, with nearly all cats studied before day 40) may have been from cats which had reached a CC plateau.

Comparison of this study's CSS with Kessler and Turner (1997) (Figure 2.11) shows that the CSS slopes are similar, with a plateau reached at around 2.75, a little below weakly tense. Kessler and Turner's study started off with a far higher CSS (around 4.75) during the first day of caging, though this may be due to different shelter conditions and possible differences in interpretation of the written cat stress score.

McCobb *et al.* found a mean CSS of around 3.01, with a very shallow decrease over time. This study's results presumably differed from others for the reasons given above for CC ratios.

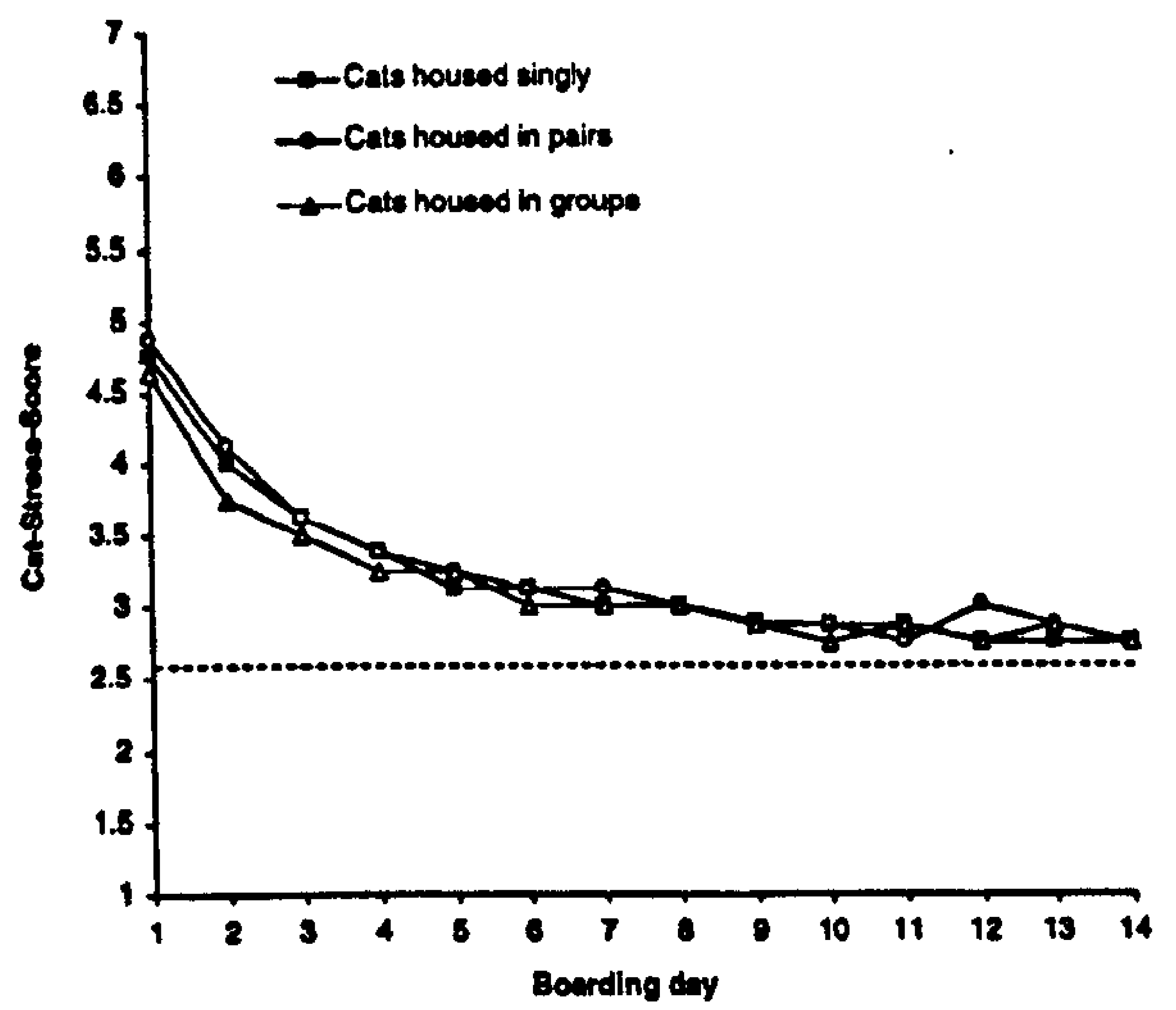


Figure 2.11 From Kessler and Turner (1997). "The development of stress (daily median CSS) in animals housed singly (N = 60), in pairs (N = 40) and in groups (N = 40) during the first two weeks in the cattery. The dotted line shows the overall median CSS of control animals in the shelter (N = 45)."

2.4.4 Differences between admissions and cattery

In admissions, both CC and CSS decline to plateau in around 5 days, which would suggest that they are both measuring the same internal state, or two states that correlate. However, in the cattery, while CC again takes 5 days to decline to plateau, CSS declines to plateau by the second day, so the stress is more temporary than upon initial admission. This is probably due to the relative familiarity of the cattery to admissions, the increased retreat opportunities it provides, and the fact the cat has not recently been removed from a home territory it may have occupied for many years. The stress of moving may mostly be due to the novel cage and lack of familiar smells in that cage (though cats were frequently moved over with their existing bedding).

CSS was the only variable different from day 8 in admissions to day 1 in the cattery (section 2.3.3), with its cattery value higher than admissions. This suggests that the adaptation to the cattery affects behavioural signs of stress more than the physiological ones. This could possibly be due to the cattery being a few degrees colder than admissions, which may affect CSS, though the plateau for admissions is similar to that of the cattery, and no significant difference was found between the two.

It could also be that the HPA axis is already less responsive as a result of the acute stress of admission and/or chronic stress from a week or more of confinement.

The lack of change in approach test variables suggests that other behavioural signs in general have reached a plateau by the time of entry to the cattery. It may be that the HPA axis takes a little time to 'wind down' in comparison to behavioural measures, which can change near-instantly. There was no detectable difference between plateau CC and CSS in the admissions and cattery (section 2.3.4), and the means are quite close, so despite having larger cages and more retreat possibilities, the cattery does not seem to result in less stressed cats than admissions, as judged by CSS. McCobb *et al.* (2005) note problems with the CSS due to assigning a low score to sleeping cats. The author's interpretation of the CSS allowed scores of 3.0 to sleeping cats, so should have avoided this problem, although it was noted that it was sometimes difficult to decide between a score of '2' or '3' for sleeping / resting cats.

Based on the above, moving cats from cage to cage appears to be a significant stressor and should be avoided for trivial reasons, but may be regarded as an acute, not chronic, stressor. The lack of any other significant changes may be due to the small sample size combined with a lower response in comparison to initial admission.

Since ten variables were tested in the one test, multiplicity would give a p=0.5 chance of one of the variables being significant purely by chance. However, from the graphs of the data (Figs 2.7 and 2.8), CSS is the variable which most obviously has a consistent change over time in both admissions and cattery, which ameliorates this.

2.4.5 Correlations between variables (individual level)

The most notable difference between admissions and cattery results is *back*, which is positively correlated with CC in admissions, but (non-significantly) negatively correlated in the cattery. CSS and *back* also has the same change, being (non-significantly) positively correlated in admissions, but negatively in the cattery. This may be due to differences in admissions and cattery caging, or due to a difference in time – although admissions cats may show avoidance behaviours when stressed, cattery cats may have become used to shelter staff and attempt to use contact with people to alleviate their stress.

CC and CSS positively correlate with each other although the variables they each correlate with differ. The observed differences between CC and CSS may be due in part to the small sample size reducing the power of the analysis, but also the differences between them – CC is a physiological measure of stress and positively correlates with levels of alertness, so may measure 'excitation'. CSS is a behavioural measure and correlates with *Affiliative*, one of the groupings with the most obvious analogies to human emotions (it also correlated with *Fearful*, though the two measures overlap) – this fits with CSS measuring behaviours which indicate stress and emotion in humans, such as increasing ventilation rate (without exercise), dilated pupils and tense muscles. Alternatively, it may correlate well with cats' attitudes to shelter staff – initially fearful, then increasingly affiliative.

The correlations in admissions suggest that cats at their higher levels of stress (as judged by individually high CC and high CSS) will be more alert, spend more time at the back of the cage, and be less affiliative when approached. Such behaviour would tend to make them less attractive to potential adopters (Vandenbussche 2001). The correlations in the cattery suggest that cats with individually high CSS will have personally high levels of *Ignore*, and low levels of *Back*. Although non-significant, there is also a positive correlation between CSS and *Affiliative* with $p = .087$. As discussed in 2.3.5, such behaviour may make the cat more likely to be rehomed. Combined with the lower CSS in the cattery, these observations suggest that 'stress' in admissions is acute and causes cats to be more wary of the environment in general, including people. 'Stress' in the cattery is more chronic and may involve being bored and wanting company as much as being fearful of the environment.

As discussed in '*Differences between admissions and cattery*' above, multiplicity may be an issue, as no correlation was significant in both admissions and the cattery. However, all three data sets had similar coefficients for many of the significant correlations in the 'all' dataset (CC and CSS, and CC and *Alert*), which suggests that non-significance was due to a low number of days tested. Of those which changed (CC and *Ignore*, CSS and *Affiliative*, and CSS and *Back*), the non-significant correlations for CSS and *Affiliative* in the cattery, and CSS and *Back* in admissions were close to significance at $p < 0.1$. The other two correlations CC and *Ignore* and CSS and *Ignore* were both significantly correlated with *Ignore* in the cattery dataset, which strongly argues that there is a real relationship present.

2.4.6 Correlations between intercepts and slopes between cats (population level)

Overall, in admissions, cats with initially high CSS also had high levels of *Ignore*, and low levels of *Post* and *Purr* – cats which were most stressed on the first day were most likely to *Ignore* the observer, and least likely to make a postural movement or to purr. In the cattery, cats with high levels of CC tended to have high levels of *Fear*, and cats with the fastest decrease in CC had the fastest increases in *Purr*.

The positive correlation between the intercepts for CC and *Fear* seem at odds with the negative correlation between CC and *Back* shown in the cattery in section 2.35, though the latter is partly based on chronic stress, rather than extrapolated day 1 data in this test, which represents acute stress.

2.4.7 Overall discussion

Both CC and CSS decrease over time after admission to the shelter, as does *back*. *Affiliative, postural* and *purr* all show positive slopes. This suggests that stress (as measured by CC and CSS) causes a high performance of *back*, and a low performance of *affiliative*, *postural* and *purr*. Similar (though non-significant) slopes for CC and CSS are found upon admission to the cattery.

The results from the correlations broadly indicate that some measures covary within individual cats as well as across the entire sample, though the exact correlations change as the cats pass from acute to chronic stress, and many of the variables with significant slopes have no significant correlations. Some variables without significant slopes had the most correlations – for example, *alert* positively correlated with CC in admissions, and *ignore* positively with both CC and CSS in the cattery.

For data from the acutely stressful phases (admissions correlations), there were signs that high levels of stress (both within-cats and between-cats) would tend to make cats less homeable (e.g. CSS negatively correlates with *Affiliative* within cats) and indeed behaviours included in the CSS itself (such as fearful behaviours) make cats with higher CSS less likely to be rehomed (Vandenbussche 2001). However, most of these correlations do not have particularly high coefficients. In the chronic phase (cattery) this was not evident, though the smaller N made any significant correlations less likely. Of the correlations that there were, CSS and *Ignore* correlated within cats, but CSS was also negatively correlated with *Back*, which would tend to make cats more attractive to the public. This suggests that either chronic stress may be less limiting to homeability, or that the cat's own personality may play a greater part in how it interacts with humans, as will the cat's learned emotional reaction to the public.

One of the most interesting set of correlations is that between CC and CSS – within cats, they are positively correlated, which suggests they are both measuring one underlying internal 'stress' state (though they may well be measuring different things which happen to co-relate). Between cats, however, cats which have a fast decrease in CC have a slow decrease in CSS and vice versa which suggests that there may be a cline of differing strategies for coping with stress – a more pronounced behavioural response but a reduced physiological one versus high HPA activation but fewer behavioural signs of stress. Other evidence from the variable correlations suggests that CC may measure 'excitation', while CSS may measure a more emotional response.

2.4.8 Welfare implications

Measures of stress (CC and CSS) decrease to a plateau 5 or 6 days after entry to admissions. From CSS, the cats are 'weakly tense', which is considered acceptable over a temporary (two-week) period by Kessler and Turner (1997), and indicates that most cats have to a large extent adapted to the shelter in this time.

Movement from place to place within the shelter may be viewed as an acute stressor for most cats, with behavioural measures of stress returning to baseline within a day in most cats. Physiological measures decline over a timescale similar to initial entry to admissions, however. On the basis of this, the author recommends that cats should not be moved from cage to cage within the shelter for trivial reasons, though the stress of the move is not so much as to preclude movement for important ones.

As mentioned above, behaviours linked to high levels of CC and CSS will make a cat less attractive to potential adopters, though this may not be obviously manifest in a chronically stressful situation. Reducing a cat's stress caused by admission to the shelter will therefore not only improve its welfare, but may also tend to reduce the time it spends in the shelter. Shelters commonly have long waiting lists of cats to be admitted, some of which may be euthanased as a result of not being admitted to the shelter. Euthanasia is perceived as a major welfare issue by owners, and since some cats are being admitted due to housing problems such as aggression to/from other cats or dogs, some cats' welfare may be poor while waiting for admission. Reducing cats' stress while in the shelter may help to improve throughput of cats and reduce waiting lists (though note that admissions cats in UK shelters are rarely seen by the public).

Some shelters routinely euthanase cats after they have been at the shelter for a certain period of time, or if it is felt that they will be difficult to rehome. The McCobb *et al.* (2005) study was conducted in North America where euthanasia is more common than the UK, with 31% of their random sample eventually euthanased compared to 56% rehomed. There was no significant difference between CSS or CC for cats which were euthanased and those which were adopted, though there was a trend for cats with very high CSS (4.5 or higher) being more likely to be euthanased.

Whether the cat *ignores* humans outside its cage or not correlates reasonably well with CC and CSS in the chronic phase. This emphasises that cats which sit at the back of the cage and don't respond in an active way to staff may be the ones in most distress.

Chapter 3: Experiment 2 - Effects of providing cats with boxes or extra social contact

3.1 Introduction

A range of studies, including Chapter 2, have shown that domestic cats experience a variable period of acute stress upon entering a novel environment such as a rescue shelter or quarantine cattery (e.g. Kessler and Turner 1997, Rochlitz *et al.* 1998a). Chapter 2 showed that urinary CC ratio and CSS are positively (but weakly) correlated within cats, suggesting that they are both influenced by a single internal state of 'stress'. Differences between cats in rates of change in these two measures suggest different 'strategies' in coping with stressful situations. Experiment 2 uses these measures and others to assess the efficacy of environmental manipulation for cats in the shelter environment.

From the studies above, and that by Carlstead *et al.* (1993), certain stressors appear to increase the motivation of some cats to hide or conceal themselves. Previous small scale studies have used the CSS to investigate the effect of providing boxes to cats entering shelters, and found significant differences between cats provided with boxes and those with open beds (Jackson and Casey unpublished data, Kry 2003). Hiding places such as boxes and shelves are greatly utilized shortly after admission, their use dropping over time (e.g. Rochlitz *et al.* 1998a).

Some shelters are unwilling to give cats boxes, as they believe that a cat hiding in a box is less likely to be adopted than a cat which does not have the facility to hide. As a result, many shelters will give cats hiding places but only if they appear particularly fearful. How much the visibility of cats to the public is affected by boxes is therefore of interest.

Social contact with humans also appears to be an important enrichment for most domestic cats - a study on serum cortisol by Carlstead *et al.* (1992) suggested that venupuncture may have been a rewarding experience for 4 out of 8 laboratory cats due to the human contact involved. The need for human contact is likely to vary between cats, due to genetic factors (McCune 1992), differences in socialisation (Kessler and Turner 1999) and previous experiences. It is also likely that while the motivation to hide decreases over time after admission, the need for social contact remains or may increase with time.

Shelters vary a great deal in the amount of contact that each cat receives with staff. Generally, staff in shelters have little time for contact with cats that is not directly related to cleaning or feeding routines. For socialized cats coming into the shelter from a household environment, this limited contact may cause frustration and stress. To the author's knowledge, neither the effect of this limited social contact, nor the impact of increasing the contact, have been examined to date.

The aim of this study is to use measures similar to those in Chapter 2 to test how the provision of boxes and/or social contact affects behavioural and psychological parameters of stress in shelter cats.

3.2 Method

3.2.1 Subjects

Cats were studied at the Bath Cats' and Dogs' Home, an RSPCA shelter in Claverton Down, Bath, UK. All cats were single-housed, in good condition and free from serious illnesses during their observation. Cats less than 1 year old or over 15 years were not included in the study. The cats were a mixture of ages, sexes and owned / strays. Cats were observed over the first 7 days they were at the shelter, and also on day 14. Over a hundred cats were observed, though only 75 cats provided sufficient data for inclusion in the final analysis. The study was carried out from the 7th January 2004 until 10th May 2004.

3.2.2 Housing

Upon entry to the shelter, cats were put into the admissions area of the cattery unless obviously suffering from disease. The cattery building consisted of 44 pens, in two rows of 22 along either side of a corridor (Fig. 3.1). At one end was the staff-only offices / food preparation / cleaning area, at the other was an entrance for members of the public. The 14 pens closest to the staff only area formed the admissions area, while the 30 pens farthest away formed the homing area. The public were allowed into the homing area, the admissions area being roped off. After 7 days, cats were moved from admissions into the homing area so long as there was space and they were free of disease. In practice, due to a lack of space in homing, around one third of cats studied were moved up to homing later than day 7.

Fig 3.1 Bath RSPCA cattery

The pods (pens) measured 0.87x0.75x0.89m (WxDxH), and the bases were 0.9m off the ground (Fig 3.2). The inside surfaces and back wall were white plastic, and a clear glass door 0.5m wide and 0.7m high formed most of the front of the cage, through which the occupant could see cats on the opposite side of the corridor. A small corridor through the back wall (0.21 x 0.29 x 0.30m, WxDxH) led to a cat flap leading on to the outside run. The cat flap was offset 0.2m away from one side wall. A blanket was placed at one back corner of the pod, furthest from the cat flap, and a litter tray was placed at the other. Food and water bowls were at the front of the pod. In addition, each pod contained a few toys but was otherwise empty.

Fig 3.2 Bath pod, with boxbed

The outside runs were fully covered. They were 1.3 x 1.6 x 2.1m (WxDxH), floored with concrete (Figs 3.3 and 3.4). The back wall was also of concrete. The front was a metal grille that could not be opened, through which the public could see the cats. The roof overhung the grille and kept out most of the rain. The cat flap opened out onto a plastic shelf 0.6m long against the back wall and 0.3m deep. A ramp led from the shelf to floor level. A windowed door in the back wall of the run allowed staff access from the inside of the cattery, and allowed the public to view outdoor cats from inside the cattery. The side walls were concrete towards the back of the cattery, but were glass towards the front, allowing cats to see and be seen by neighbours. Cats were observed to interact through the glass, either peacefully by sniffing through the gaps, or aggressively, including trying to attack through the glass.

Cats started off with their litter tray placed inside the pod. It was moved outside under the ramp once the cat was thought to be ready to use an outdoor tray.

Fig 3.3 Bath run with (left) boxbed, (right) box.

Fig 3.4 Bath cattery, outside view.

Admissions and homing pods and runs were identical. The public were allowed to go close up to the grille at the front of homing runs, but were kept a few feet away from admissions runs. The left hand side of the cattery overlooked a wide path which was one of the main routes used by the public and staff through the site, the right hand side overlooked a path which was only used by people viewing cats. Both paths had a fence on the other side, behind which were dog runs. The sound of dogs' barking could be heard throughout the day.

The cattery has two permanent staff, and one member of staff who swapped between the cattery and the other cat housing blocks on site. On weekends, volunteers often replaced one of the permanent members of staff. The daily routine was the same for all 3 blocks. Staff started at 0830 with feeding the cats. After this, litter trays were changed, and then the pods were cleaned. This involved removing the blanket so the floor could be washed, and timid cats often retreated outside while cleaning took place. Cleaning usually finished by 1045, and the public was let in at 1100. Litter trays were changed during the day if they were used. The shelter was closed to the public at 1600, and staff then fed the cats and changed the litter trays. The shelter staff generally left by 1700, and the lights were turned off.

3.2.3 Protocol

Cats were observed every day from day of admission (day 1) until the day they were due to move up to the homing section of the cattery (day 8 or 9). Days 1-7 form a continuous record for each cat. Data from days 8 and 9 were not used for analysis as many cats did not actually move up on those days. Cats were also observed on day 14, one week after being moved to homing. Since the actual day for moving might be delayed, and some cats were due to be rehomed before day 14, 'day 14' data included data from days 12-16, though 78% was from day 14 (mean 14.2, Standard Deviation 0.81). Data were taken from no earlier than the 4^{th} day after moving pods.

The number of cats studied varied from day to day as cats arrived and got taken off the study due to illness, being rehomed, etc. The maximum number of day 1-7 cats the author could comfortably study was 8, though up to 10 were occasionally observed on a single day.

Cats were placed upon entry into four groups:

No box, No contact (hereafter, Nbox, Ncontact or NN)
Box, No contact (Box, Ncontact or BN)
No box, Contact (Nbox, Contact or NC)
Box, Contact (BC)

"Nbox, Ncontact" cats (NN) were the 'control' and treated according to normal shelter guidelines, with the addition of a 'boxbed' (see NC, below).

"Box, Ncontact" cats (BN) were given a box in addition to their normal cage furniture, and were otherwise treated normally. The boxes measured 0.26 x 0.36 x 0.26 (WxDxH) and were placed in the back corner (Fig 3.5). The boxes had one of the long sides removed so the cat could still be visible to the public, and for scoring the CSS. The boxes also had no base to them and rested directly over the blanket already present. This allowed the box to be easily removed for access to the cat if necessary. An additional box was placed in the run, in the back corner under the shelf. This box had the flat base retained so the cats were thermally insulated from the concrete to some extent. The boxes were single ply cardboard (Transatlantic Plastics Ltd, Southampton UK) and were not strong enough for the cats to sit on the roof of the box. They were designed such that the cat was more hidden in the pod than normally allowed, but could still be seen readily by the author for behavioural observations, the staff when making their regular checks, and the public for rehoming purposes. The boxes stayed with the cat throughout the 14 days, unless urinated / defaecated on or clawed to pieces, in which case they were replaced.

"Nbox, Contact" (NC) cats had normal cage furniture and had extra social contact from the author twice a day, as detailed in 3.2.10 ("*Social test*"). Having a box constrains the cat's use of the space inside its pod - it cannot stretch out from the front to the back across the blanket for example. To reduce this difference between Nbox and Box groups, Nbox pods had a boxbed (Fig. 3.2) - a box with the roof and most of the walls cut away to leave a 5cm wall. This provided minimal extra concealment for the cat while constricting its use of space. In the outside run, the flat bottom of a box with a 5cm wall was placed instead of a box.

"Box Contact" (BC) cats had both a box and extra social contact.

The boxes for Box groups, and boxbeds for Nbox groups, were put into the cage before the cat was. This was important as cats often pick a hiding place immediately after entry and then remain there even if a box is subsequently added. This preference often lasts for the duration of the first day before many cats transfer their preference to the box, and sometimes longer.

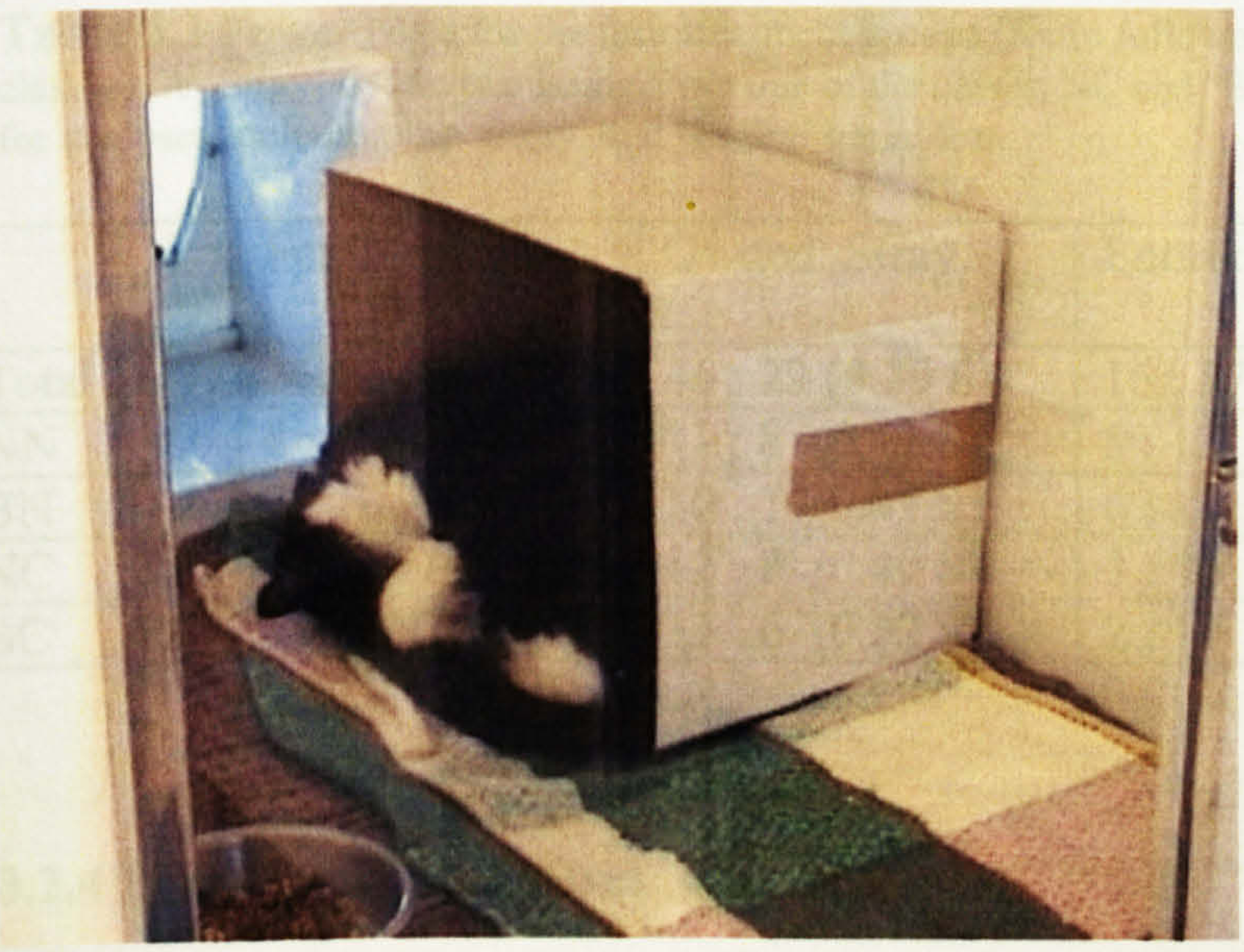


Fig 3.5 Pod belonging to cat in Box treatment group.

One cat was entered into each of the four groups before replication of a group occurred i.e. the order of groups for the first 4 cats was randomised, then the order for the second four, and so on. This ensured that all groups were more or less equal in size, and spanned the length of the study. In practice, cats that dropped out due to illness or being rehomed made the groups slightly unequal, and the distribution of sexes, being stray or owned, age, and neuter status were all a little uneven. To redress this, cats which entered the shelter during the latter half of March onwards were assigned to a treatment group to restore the balance of all these factors as far as possible. The final distribution per treatment group is in Table 3.1. Demographic data recorded were: Age (in years), 'Sex' (male = 0, female = 1), 'Entire' (neutered prior to entry = 0, entire = 1) and 'Stray' (Owned = 0, Stray = 1). The mean age for all cats was 4.9 years. For NN, BN, NC and BC respectively, it was 3.8, 4.9, 5.3 and 5.6.

The initial sample of 91 cats that were observed upon entry declined to 75 cats by day 7 as a result of stray cats being claimed, becoming ill while at the shelter, etc. Since the analysis looks at cats over the course of the first seven days, comparisons between days should not be affected by cats leaving. Therefore, only the 75 cats which were present for all seven days were kept in the final dataset. This reduction did not increase the SE of the data appreciably.

Table 3.1 Personal data for the four treatment groups (Ratio followed by proportion of one of the classes). A 'Stray' datum is missing from one of the cats in NC and 'Entire' from one in NN, so N = 74 for analyses including the 'Stray' and 'Entire' variables.

	N	Female: Male	Owned: Stray	Entire: Neuter	Male Neut : M Ent : Fem Neut : F Ent
Total	75	36 : 39 (0.48 F)	46 : 29 (0.39 S)	16 : 57 (0.22 E)	27 : 11 : 30 : 5
NN	19	8 : 11 (0.42 F)	11 : 8 (0.42 S)	5 : 13 (0.28 E)	7 : 3 : 6 : 2
BN	17	7 : 10 (0.41 F)	10 : 7 (0.41 S)	6 : 11 (0.35 E)	5 : 5 : 6 : 1
NC	20	10 : 10 (0.50 F)	12 : 8 (0.40 S)	3 : 16 (0.16 E)	8 : 2 : 8 : 1
BC	19	11 : 8 (0.58 F)	13 : 6 (0.32 S)	2 : 17 (0.11 E)	7 : 1 : 10 : 1

3.2.4 Physiological measures

Urinary cortisol was measured using the same techniques as Chapter 2, based on the technique of Carlstead *et al.* (1993) and Rochlitz (1998a). Briefly, urine samples were collected by leaving a two tiered litter tray in place of the usual litter tray. The upper tray was perforated with small holes and contained plastic non-absorbent litter so that urine drained through to the lower litter tray where it was collected. Urine was collected daily on days 1-7, and again on day 14. Trays were changed in the morning between 0900 and 0930. If a tray was urinated in during the day, the top tier was replaced so the cat had clean, dry litter again. After urine collection, trays and litter were rinsed well with water and dried overnight. Litter was reused for approximately a fortnight before being replaced with new litter.

It was initially intended to have trays inside the pods, as this worked in the pilot study and would keep conditions the same for all samples. However, it became clear that many cats would urinate on the floor outside (or on the box or boxbed) rather than use an inside tray. Because of this, trays were left inside as long as the cat readily used them, but were moved outside if necessary.

To combat the large day to day variation found in experiment 1, each cat had the urine from days 2 and 3 pooled, as was urine from days 6 and 7. Day 14 urine was left unpooled. Urine from days 1, 4 and 5 was not collected, though the two tiered litter trays were still used to keep conditions similar. Day 2 urine, after collection on the morning of day 3, was kept refrigerated overnight at 4°C. It was then mixed with day 3 urine the following morning, and a sample taken of the mix. This sample was frozen on site at –20°C. Pooled urine from days 6 and 7 was treated similarly. Day 14 urine was sampled and frozen after collection. Urine was analysed for cortisol and creatinine concentrations by RIA at Axiom Veterinary Laboratories (Teignmouth, UK).

The volume of urine was also noted so that the total volume of cortisol produced by the cat could be calculated. It was hoped that this might bypass the need to use creatinine as a calibration tool. Creatinine is a by-product of muscle metabolism, so if stressed cats tend to be more (or less) active, creatinine might not be suitable as a calibration for urine volume.

3.2.5 Behavioural measures - schedule

Five sets of measures were taken: Maintenance behaviours, scans, CSS, social tests and approach tests. The first 4 were conducted on days 1-7 and 14 according to the schedule (Table 3.2). The approach tests were conducted on days 3, 7 and 14 only.

The schedule for day 14 was different, as CSS and social tests could not be performed during the daytime when the public was present. Scans were taken throughout the day as usual, though CSS and social were not. An extra scan, CSS and social test were all conducted at 1630 once the public had left. The time taken to complete these tests depended on how many cats there were (usually no more than 30 minutes), though 5 minutes was always left between tests.

The schedule for day 1 was also different as many cats arrived in the afternoon. No tests were conducted until the cats had spent at least 2 hours in their pod, as CSS declines most quickly during this time (McCune 1994). This meant that many cats would have no data for day 1 unless extra tests were introduced. Scan 5 and CSS 3 were conducted for all d1 cats, though social 3 only if the cat had not had its daily quota of social contact yet that day (see "*Social*" 3.2.10).

Table 3.2 Daily schedule, days 1-7, day 14

Time	**Test: days 1-7**	**Test: day 14**
1050 (before public in)	Approach test (d3, d7 only)	Approach test
1100	Scan 1	Scan 1
1115	CSS 1	
1200	Scan 2	Scan 2
1215	Social 1 (NC and BC only)	
1330	Scan 3	Scan 3
1345	CSS 2	
1430	Scan 4	Scan 4
1445	Social 2 (all cats)	
1630 (public no longer in, after cats fed)	Scan 5 (d1 cats only)	Scan 5
	CSS 3 (d1 cats only)	CSS 3
	Social 3 (d1 cats only)	Social 3

3.2.6 Maintenance

For each day (starting and finishing at around 0900) whether each cat fed, urinated or defaecated were recorded, along with whether the cat destroyed part of its cage furniture (Table 3.3). Urinate had a separate 'morning' category (0830 – 0900) as urine produced then still counts as the previous day's for purposes of sample collection. For analysis, *feed day* and *feed o/n* were conflated to create *feed all* with a score of '1' if the cat fed at all in those 24 hours. *Urinate all* was similarly created from *urinate day*, *urinate o/n* and *urinate morning*.

Table 3.3 Maintenance measures; recorded as presence '1' or absence '0'

Measure	*Definition*
Feed_day	Fed between 0830 and cattery close. Classed as '1' if some food missing from food bowl, or if bowl taken away because empty. Otherwise, '0'.
Feed_o/n	Fed overnight
Feed_all	Fed between 0830 and the same time the following day
Urinate_day	Urinated between 0900 and 1700. Classed as '1' if wet litter in tray or urine in lower tray. Otherwise, '0'
Urinate_o/n	Urinated between 1700 and 0830
Urinate_morn	Urinated between 0830 and 0900
Urinate_all	Urinated between 0830 and the same time the following day
Defaecate	Defaecated between 0830, and 0830 the next day
Upset cage	Evidence of some aggression / escape attempts – bowls overturned, blanket rucked up, etc.
Demolish cage	As above but very excessive aggression / escape behaviour.

3.2.7 Scan test

Each scan involved the author standing in front of a pod, recording the position and behaviour of the occupant, ending in scoring the CSS. The various measures and categories for scoring are listed in Table 3.4. For each set of scans, the order of cats in the scan was randomised with the following criteria: cats in admissions were blocked together, as were cats in homing. If a cat due to be scanned was opposite or directly next to the previous scanee, it was left until all other cats in the block had been scanned. Cats which were grooming or had members of the public or staff close to them were also left until the end. Waiting for the cats to be undisturbed sometimes meant that the scans took longer than the 10 minutes allotted, though this was rare. After each scan, the author retreated to the end of the cattery for at least 30 seconds

Table 3.4 Scan measures recorded

Measure	Definition
InOut	In pod = 1 Out in run = 2
hf/bf	Head closest to front of pod = 1 Body closest to front of pod = 2
Hide[1]	Not hiding = 1 Attempting to hide = 2
Face	Cat facing front (towards observer) = 1 Facing side wall = 2 Facing back wall =3 Facing outside (through catflap) = 4 Facing into box = 5
Behaviour[2]	Active / standing = 1 Resting (sitting or lying) alert = 2 Lying (ventral or side) eyes closed attentive, or can not see if eyes open or shut = 3 Lying (ventral or on side) eyes closed relaxed = 4 Other = 5
Exposedness[3]	In open part of cage, or on blanket (not in box or corridor) = 1 In box/corridor/under ramp (b/c/r), head exposed = 2 In b/c/r, head inside b/r/c but looking out = 3 In b/c/r, head and body inside, not looking out = 4 In b/c/r, head and body hidden and pressed close to side = 5
Position In pod	 Front of cage = 1 Back of cage, not in box / on boxbed = 2 Back of cage, in box or boxbed = 3 In corridor = 4
Out run	Front of cage (floor) = 1 Back of cage (floor) = 2 Under ramp = 3 On shelf = 4 In box or boxbed = 5
CSS	CSS after scan test.

Notes 1) *Hide*: "Attempting to hide = 2" was scored regardless of success at hiding – cats attempting had flattened postures and appeared to be trying to hide behind litter trays, blankets, in the box or squeezed against back wall as far as they were able. Cats merely lying in the box were not counted as hiding.

2) *Behaviour* was coded such that classes 1-4 comprised an ordinal scale of 'alertness'.

3) *Exposedness*: Having the box present allows the cat to become less exposed more easily. *Exposed* recorded how much attempt the cat was making to hide, as this could be converted to how exposed the cat actually was (*vice versa* not being possible). Therefore, *Exposed* measured the amount of the cat that was in the hiding place (box, corridor, under ramp) rather than how easily it could be seen. An exposedness of 5 was roughly equivalent to "attempting to hide = 2".

before walking up to the next cage, to avoid (as much as possible) the cats' positions being a direct response to his presence. Since there would nonetheless be some effect, the author walked up and down the row of pods / pens and then waited for 30s before starting observations on the first cat to keep it as similar as possible to subsequent scans. Measures of position and behaviour were recorded based on the very start of the scan (i.e. as close to an instantaneous sample as possible).

This took around 30s, after which the CSS was taken. The CSS will have included any effect of the author's presence on CSS. The CSS was recorded slightly differently from Chapter 2, to explicitly include the cat's reaction to the observer: see "CSS", below.

3.2.8 CSS

The CSS measured during the scans (above) cannot help but include any effect of the observer on the cats' stress. Since some cats in this study were receiving more social contact with the observer than others, this might cause a difference in the CSS between the two groups unconnected to any more general lowering of CSS. To obtain unbiased records of CSS, a video camera was used to observe the cats. For each cat, a video camera was set up opposite its pod on the other side of the corridor, or outside the run. The camera was started recording, the observer retreated to the staff's quarters, waited for 2m30s, then returned to the camera and stopped the recording. The CSS was obtained from watching the final 30s of the recording at a later date. To save time, two cameras were set up on two different cats, one after the other, and then both started at the same time.

Cats were videotaped in a random order. If a cat due to be videotaped was opposite or directly next to the previous subject, it was left until all other cats in the block had been videotaped. Cats which were grooming or had members of the public or staff close to them were also left until the end. This sometimes meant that the videos took longer than the allotted 45 minutes, though this was rare. Quite often (around 2 cats in every 8), cats would move out of the camera's view, or staff would commence routine tasks close to the pod, so the CSS had to be redone at the end.

The CSS (Kessler and Turner 1997) allows some room for interpretation, and the author's personal experience suggests that everyone using it does so in a slightly different way. The CSS used was changed slightly from Chapter 2, to make it more consistent for the author, and to address some problems found with his interpretation of the original CSS. Most notably half measures were added to the CSS scale. These made the CSS data better approximate a normal distribution, and clarified some inconsistencies. This new CSS (used for videotaped and scan CSS) is specified in Table 3.5, with changes to Kessler and Turner (1997) in blue.

3.2.9 Approach test

Any effect of increased social contact on the cats' reactions to the author in the social test might be due to their becoming habituated to him, rather than a more global effect such as reducing anxiety. To test this, cats were subject to an approach test by humans they had not met before. Members of BCDH reception and office staff who did not work with or own dogs were used for this. The timing of the approach test was determined by when most people were free (morning coffee break). The test was designed to be quick and unambiguous, so did not include any friendliness ratings. A cat was not approached by the same volunteer more than once. Only female volunteers were used to avoid any sex bias.

The volunteer approached the pod (or door leading outside in the case of cats which were outdoors), put her hand on the glass, and said 'hello cat'. She waited for 10 seconds, then opened the door, said 'hello cat' again and put her hand just inside the pod for 10 seconds, then withdrew and shut the door. In the case of cats in the outside runs, the volunteer went out into the run completely and shut the door behind herself, returning after 10 seconds. If a cat showed an unfriendly response (postural or body withdrawal, fearful or aggressive response, ears back, hiss, growl, spit) the test was ended there for the safety of the volunteer, and to avoid causing the cat unnecessary stress.

Table 3.5 Cat stress score, based on Kessler and Turner (1997)

	Body	Belly	Legs	Tail	Head	Eyes	Pupils	Ears	Whiskers	Vocalisation	Activity
2	*i*: laid ventrally or half on side or loose roll (showing belly) or sitting *a*: standing or moving, back horizontal	exposed or not exposed, slow or normal ventilation	*i*: bent, hind legs may be laid out *a*: when standing extended	*i*: extended or loosely wrapped *a*: tail up or loosely downwards	laid on the surface or over the body, some movement	closed, half opened or normal opened	normal	half back (normal) or erected to front	lateral (normal) or forward (normal)	None (Meow if eliciting affiliative contact)	Restful sleeping, resting, alert or active, may be playing
2.5	*i*: laid ventrally, sitting or rolled *a*: standing or moving, back horizontal	Not exposed, normal ventilation	*i*: bent *a*: when standing extended	*i*: close to the body, not twitching *a*: tail up	Over the body, some movement	Normal opened or closed	normal	Half back (normal) or erected to front	Lateral (normal) or forward	None (meow if eliciting affiliative contact)	Resting, awake or active
3	*i*: laid ventrally, sitting or rolled *a*: standing or moving, back horizontal	not exposed, normal ventilation	*i*: bent *a*: when standing extended	*i*: on the body or curved backwards or close to the body, may be twitching *a*: up or tense downwards, may be twitching	over the body, some movement	normal opened or closed	normal	half back (normal) or erected to front or back and forward on head	lateral (normal) or forward	meow or quiet (Plaintive meow if eliciting affiliative contact)	Resting, awake or actively exploring Sleep
3.5	*i*: laid ventral, rolled or sitting *a*: standing or moving, back horizontal	Not exposed, normal ventilation	*i*: bent *a*: when standing extended	*i*: close to the body or on the body, may be twitching *a*: tense downward, may be twitching	Over the body, little movement	Normal or widely opened	Normal	Half back or erected to front or back or back and forward on head	Lateral (normal) or forward	Meow or quiet (Plaintive meow if eliciting affiliative contact)	Resting, awake or actively exploring Sleep
4	*i*: laid ventral, tightly rolled or sitting *a*: standing or moving, body behind lower than in front	not exposed, normal ventilation	*i*: bent *a*: when standing hind legs bent, in front extended	*i*: close to the body *a*: tense downward or curled forward, may be twitching	over the body or pressed to the body, little or no movement	widely opened or pressed together	normal or partially dilated	Erected to front or back, or back and forward on head	lateral (normal), forward or back	meow, plaintive meow or quiet	Cramped sleeping, resting or alert, may be actively exploring, trying to escape

Approach test scoring:

1 = Approaches in the first 10 seconds (door closed)
2 = Does not approach in first 10s but does approach in the second 10s (door open)
3 = Neither approaches nor withdraws but shows friendly behaviour
4 = Neither approaches not withdraws, no friendly behaviour is shown.
5 = Does not withdraw in first 10s but does withdraw in the second 10s (door open)
6 = Withdraws in the first 10s (door closed)

3.2.10 Social

The social test started off with an approach test, of the same format as that used by the staff volunteers. The author approached the pod (or the door leading outside if the cat was in the run), put his hand on the glass and said 'hello cat'. He waited for 10s, then opened the door, put his hand just inside the cage and said 'hello cat' again, and waited for 10s. In the case of outdoor cats, he went into the run and shut the door behind himself. After this, he then interacted with the cat according to its preference. For cats in the 'Contact' treatment group, this lasted for 10 minutes, for those in the 'Noncontact' group, only for a further 40s. Measures taken during this social test are in Table 3.6

Table 3.6 Measures taken in the social test

Measure	Definition
Approach test	Scored as '*approach test*' above
Social 1st minute	Friendliness during the 1st minute
Social 10th minute	Friendliness during the 10th minute

'Friendliness' was scored on a 1 to 6 categorical scale, with 1 being 'very friendly' and 6 being 'very unfriendly' (Table 3.7). The scores were based on the Human Approach Test by Kessler and Turner 1997, as defined by Kessler (pers. comm.), though modified for consistency when scored by the author.

Table 3.7 Friendliness scores and descriptions of corresponding behaviour

Score	Definition
1	"very friendly": cat is orientated towards, and shows friendly behaviours[1] to the observer for the duration of the minute. Shows no fearful[2] or aggressive[3] behaviours.
2	"quite friendly": cat is orientated to, and shows friendly behaviours to, the observer during the minute, but not continuously – some behaviours are not directed towards the observer, e.g. investigating other parts of the cage, looking away from the observer, grooming. No, or few, aggressive behaviours. Some friendly cats which were nervous fall into this category as they are also focusing on their environment. Cats which try to escape past the observer by showing friendly behaviour and then slipping past are also put into this category.
3	"slightly friendly": cat shows very little friendly behaviour. No aggressive behaviours. Orientated towards the observer during some of the minute. Typically, a cat which does not approach but meows or purrs at the observer. Generally nervous cats which seem to enjoy stroking.
4	"neither": cat shows no friendly or aggressive behaviours. May or may not be orientated towards the observer. Typically, a cat which either does not approach, or ignores the observer. Tolerates stroking but does not elicit it. The occasional cat which shows a mix of friendly and unfriendly behaviours was put into this category.
5	"quite unfriendly": cat is orientated to the observer during the minute. None, or few, friendly behaviours. May be some unfriendly behaviours. Will show some fearful behaviours. Typically, a cat which looks nervous upon being approached but does not flee or react aggressively. May tolerate stroking, but does not elicit it.
6	"very unfriendly": cat is orientated to the observer the entire minute. No friendly behaviours. Will show unfriendly or fearful behaviours. Typically, a cat which withdraws into the other part of the accommodation (pod or run) and/or reacts aggressively upon approach.

Notes:
[1] "friendly" behaviours – Tail up and may be vibrating, rubbing against observer or cage furniture, greeting vocalisations, ears forward, may be purring.

[2] "fearful" behaviours – Ears back, staring, lowered posture, postural or body withdrawal. Tail close to body.

[3] "aggressive" behaviours – Growling, yowling, hissing, may strike out with paw, staring. Nearly always combined with fearful behaviours when performed towards observer.

If a cat showed an unfriendly response (postural or body withdrawal, fearful or aggressive response, ears back, hiss, growl, spit), the observer withdrew to a distance where the cat looked comfortable, or closed the door and stood away, if necessary remaining outside the pod for the full time of the test. This was to get the cat used to his presence. Because cats frequently adapted very quickly as they became less stressed over the first few days, he continued to approach the cat at the start of the first few tests regardless of the outcome of previous tests. Only if the cat retained a *friendliness* of 5 or 6 into day 3 did he change the protocol, and no longer approached the cat (though it retained its previous score) until it seemed to change behaviour towards him.

3.2.11 Analysis

Maintenance

Chi-square tests for each variable were conducted, comparing all 4 treatment groups. Conducting these tests on every day would require the p-value to be greatly lowered to account for multiplicity, so tests were only conducted on days 3, 7 and 14. The variables tested were: *feed day*, *feed o/n*, *feed all*, ***urinate day***, ***urinate o/n***, ***urinate all***, ***defaecate all***, ***upset cage*** and ***demolish cage***.

Cortisol

Histograms of cortisol, creatinine and CC ratios for each day were all right hand skewed. The data were subject to a $\log_{10}$ transformation to make them better approximate a normal distribution. Data from one NC cat was inexplicably very high on day 6/7 and day 14, so its data were removed from the cortisol analysis. Normality tests were conducted for each days' data, for cortisol, creatinine and CC. All were NS at $p > 0.1$ apart from day 2/3 cortisol ($p = .050$) and day 6/7 CC ($p = .022$). Given the known oversensitivity of the normality tests and the multiplicity involved, these significance levels were not concerning.

Repeated measures GLM was used to look at differences between treatment groups. The initial equation had $\log_{10}$ transformed CC ratios day 2/3 and day 6/7 as the dependent (y-) variables, and had Box, Contact, the interaction between them, sex, entire, the interaction between sex and entire, and stray as fixed factors (categorical x-variables), and age as a covariate (continuous x-variable):

Transformed CC d2/3, d6/7 (day) = box|con + sex|entire + stray + age   (N = 69)

'Box|con' stands for 'box + contact + box*contact', ditto for sex|entire . Type III sums of squares were used. Terms which did not add anything significant to the model (as judged by significance of the multivariate, within-subjects and between-subjects tests) were removed, and the model retested. Box's test of equality of covariance matrices, Mauchley's test of sphericity and Levene's test of equality of error variances and Lack of fit tests were all NS unless mentioned in the text. Day 14 CC was then added as an additional dependant variable, and the model retested. As with all repeated measures GLMs, any cats with missing data from any days are excluded.

The total cortisol produced was calculated for each sample by multiplying cortisol concentration with the urine volume. The volumes of days 2 and 3 were added together to give the total volume of urine from the two days. This was then multiplied by cortisol concentration to give total cortisol secreted into the urine over the two days. Total cortisol data had right hand skew, and were square root transformed to meet the assumption of normality.

If the cat did not urinate on both day 2 and day 3, the recorded volume would not reflect the volume produced over those two days, so the following compensations were carried out to the recorded volume data: if the cat did not urinate on days 1 or 2, so d2/3 urine was secreted on day 3 and was pooled from days 1,2,3, the volume was multiplied by 2/3; if the cat did not urinate on day1 but did on days 2 and 3, then d2 volume was divided by 2 and added to d3 volume; if the cat urinated on both days 2 and 3, but only urine from one of the two days was collected, that volume was multiplied by 2. Total cortisol was subject to GLM using the same x-variables and method as above.

Approach test

Kruskal-Wallis tests for differences between all four treatment groups were conducted for all 3 test days. Mann-Whitney U tests were used to look for differences between Nbox and Box groups, and also Ncontact and Contact groups.

Ordinal regression was carried out for each of the three test days to look for effects of the treatment groups in more detail. Histograms of *approach* suggested a negative log-log function be used (which expects most data to be in the lower ranked groups). The same x-variables as for CC were used:

Approach = box|con + fem|ent + stray + age

As before, terms which did not add to the model were removed.

Approach on day 7 was subtracted from day 3 to give the change over time, and this was tested with a Kruskal Wallis test between all 4 groups, and Mann-Whitney tests between Box and Nbox, and between Contact and Ncontact groups. The change over time was also subject to ordinal regression with the standard initial equation and probit as the link factor.

Social

Firstapp

A problem with the method was that Contact cats had their first day's social in the morning, while Ncon cats had theirs in the afternoon. Comparing social tests between the two groups is difficult – the afternoon social is the second one that day for contact cats, but the first for Ncon cats. For analysis, the first social test that day was used for each cat (i.e. morning for Contact cats, afternoon for Ncon cats).

For comparisons between all 4 treatment groups, results from day's first approach test for each cat (*firstapp*) were used as above.

Kruskal-Wallis tests were performed on each day, looking for differences between all 4 treatment groups. For d14, the approach test from social 3 was used. Mann-Whitney Tests were also performed on each day, looking for differences between Nbox and Box, and between Ncontact and Contact.

To check for effects of other variables, ordinal regressions were used on days 3, 7 and 14, using the negative log-log link function. The standard equation was used initially, paring down to the best equation as before:

$$Approach = \text{box}|\text{con} + \text{fem}|\text{ent} + \text{stray} + \text{age}$$

Social 1st minute

The friendliness score from the 1st minute of social contact from the first social each day for each cat (*soc1st*) was analysed in the same way as *firstapp*.

Contact group

For comparison between NC and BC treatment groups, the median of both social tests that day can be used to increase the reliability of the test. Mann-Whitney U-tests were performed on days 3, 7 and 14 for median *approach*, median *soc1st* and median *soc10th*.

Change over time

To look at changes over time, the median of the first approach on days 6 and 7 was subtracted from the median of the first approach on days 2 and 3 (*appchange*). The same was done for soc1st (*soc1stchange*). Kruskal Wallis tests were carried out on both variables to look for differences between the treatment groups, and Mann-Whitney tests used to compare Box and Nbox groups, and also Contact and Ncontact groups.

CSS

For each day, the two CSS tests were averaged to give that day's datum for each cat (*avgCSS*).

To check that morning and afternoon data could be combined this way, morning and afternoon scans were tested with a Wilcoxon signed ranks test, for each of the first 7 days, and all seven days' data combined. All were NS at $p > 0.1$ apart from d2 ($p = .039$), in which the morning CSS was slightly higher. This might be expected as it was only the second videotaping for each cat.

Normal Q-Q plots confirmed the data fitted a normal distribution. Though there was a small left hand skew, untransformed data was used as the gain from transformation would be small, and complicates conclusions drawn from parameter estimates.

Repeated measures GLM was used to look for differences between treatment groups, analysing *AvgCSS* for days 2-7. Variables that did not contribute to the model were removed, though favouring keeping the treatment group variables in. Type IV Sums of Squares were used. Polynomial within-subjects contrasts were analysed to look at changes over time. The standard starting equation was used:

avgCSS days 2,3,4,5,6,7 = box|contact + fem|ent + stray + age

CSS 3 (late afternoon reading: see Table 3.2) on day 1 was also tested, with the same x-variables as above, as was CSS 3 from day 14.

Scan CSS

Friedman's tests for differences between the 4 scans each day were conducted, and were all NS at $p > 0.1$ apart from d3 $p = (.002)$, d6 $p = (.057)$, d7 $p = (.066)$ and d14 $p = (.032)$. The ranks tend to increase across the day, though this may be expected as afternoon CSSs were generally slightly (though not significantly) higher. The mean *ScanCSS* from each day's 4 scans was taken as that day's datum for each cat. A histogram of ScanCSS showed very slight right hand skew, though the data did not need transforming.

Repeated measures GLM was used to look for differences between treatment groups. *ScanCSS* was analysed for days 2-7 with the standard initial equation:

ScanCSS days 2,3,4,5,6,7 = box|contact + sex|ent + stray + age   (N = 75)

Analysis was as per 'CSS', above. CSS 5 data from Scan 5 was analysed for day 1, and day 14.

Scan

One variable was recoded before analysis: for *face*, measures 1,2,3 and 5 form an ordinal scale of 'facing towards' and facing further away from the front. Score '4', looking outside (through the cat flap if indoors), does not fit into this ordinal scale. Because of this, cats which were in the pod and scoring '4' were recoded to 'no data', and cats which were outside and looking out were recoded to '1', as they were facing the public who walk along the paths.

To look for differences between treatment groups, Kruskal-Wallis tests were performed on each of the 4 scans for each day (1-7 and 14) for all variables except *position*, which was tested by Chi-sq, as it was not ordinal. Since this gave 56 cells for each variable, multiplicity was taken into account with Bonferroni correction unless a definite pattern emerged.

To look at differences between individual categories in each day, Kruskal-Wallis tests were performed for days 1-7, and day 14, for each category (i.e. *face* score '1', face score '2', etc) to see if any particular category changed over time. Again, Bonferroni corrections for multiplicity were necessary if there was no overall pattern present. The results suggested that the effect of treatment groups on position was purely due to the Box / Nbox groupings, so Mann-Whitney U-tests were performed on Nbox and Box groups on *position* data.

For each variable, the proportion of time each cat spent performing each category each day was computed. Using data from all days from all cats (N = 630) these data were entered into factor analysis. After interpretation, the 5 most significant factors as decided by scree plot (which together explained 61% of the variance) were then entered into repeated measures GLMs. The only significant variables were those which were to do with exposedness. Hence, these tests had no more explanatory power than the Kruskal-Wallis tests above, and will not be discussed further.

3.3 Results

3.3.1 Maintenance

All variables tested with chi-square tests for differences between treatment groups on days 3, 7 and 14 (*feed day*, *feed o/n*, *feed all*, *urinate day*, *urinate o/n*, *urinate all*, *defaecate all*, *upset cage* and *demolish cage*) were non-significant (NS) on all days at $p > 0.1$ apart from *feed day* d3, $p = .030$. This was considered non-significant due to multiplicity.

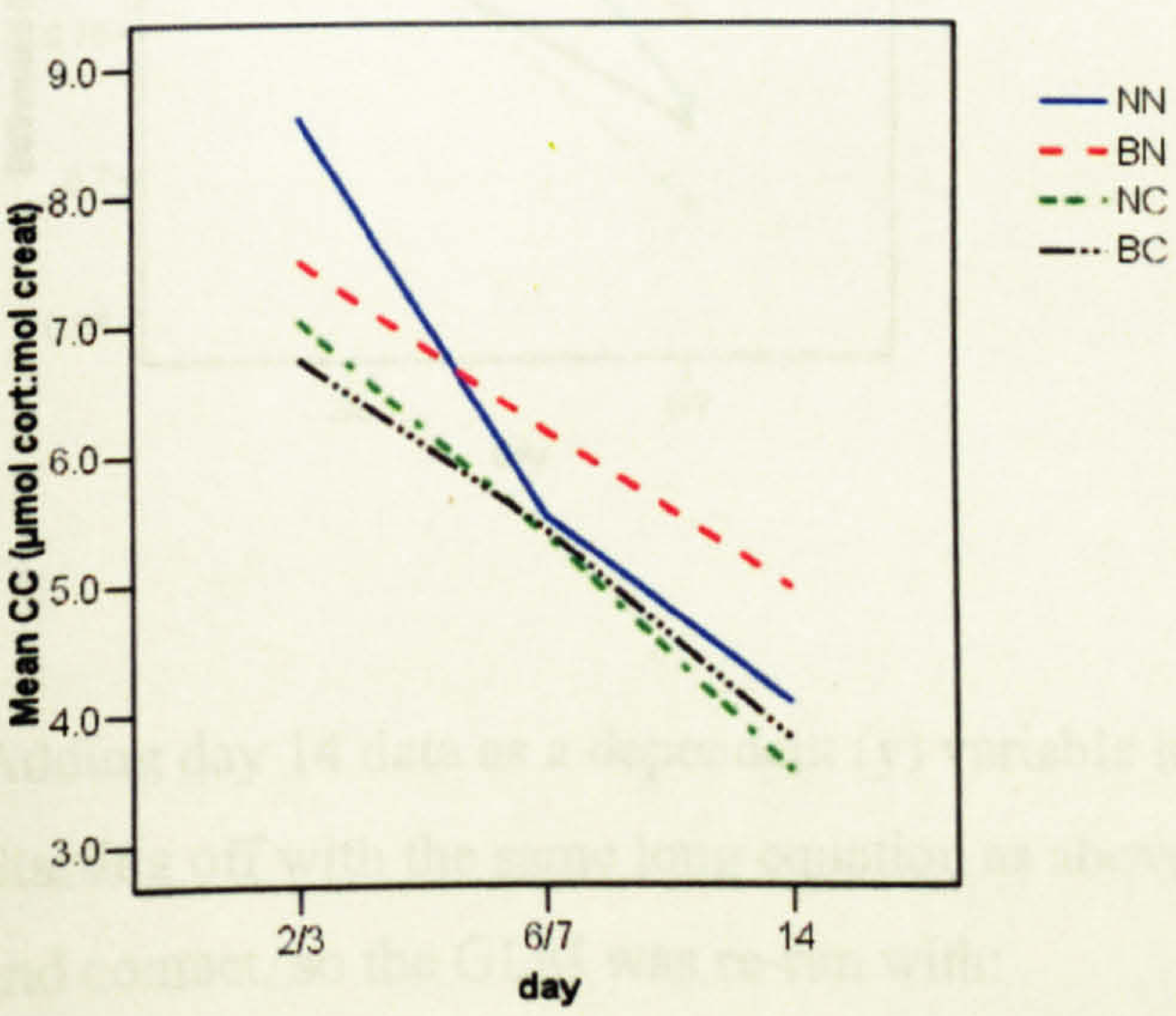
In conclusion, treatment group had no significant effect on any of the maintenance variables tested.

3.3.2 Cortisol

CC ratios

Cortisol:Creatinine ratios were obtained for N = 72 cats on days 2 and 3 (day2/3) and N=73 days 6 and 7 (day6/7). CC ratios for N = 38 cats were obtained for day 14 (Fig. 3.6).

Figure 3.6 Graph of mean CC ratios by treatment group.



The initial GLM equation was:

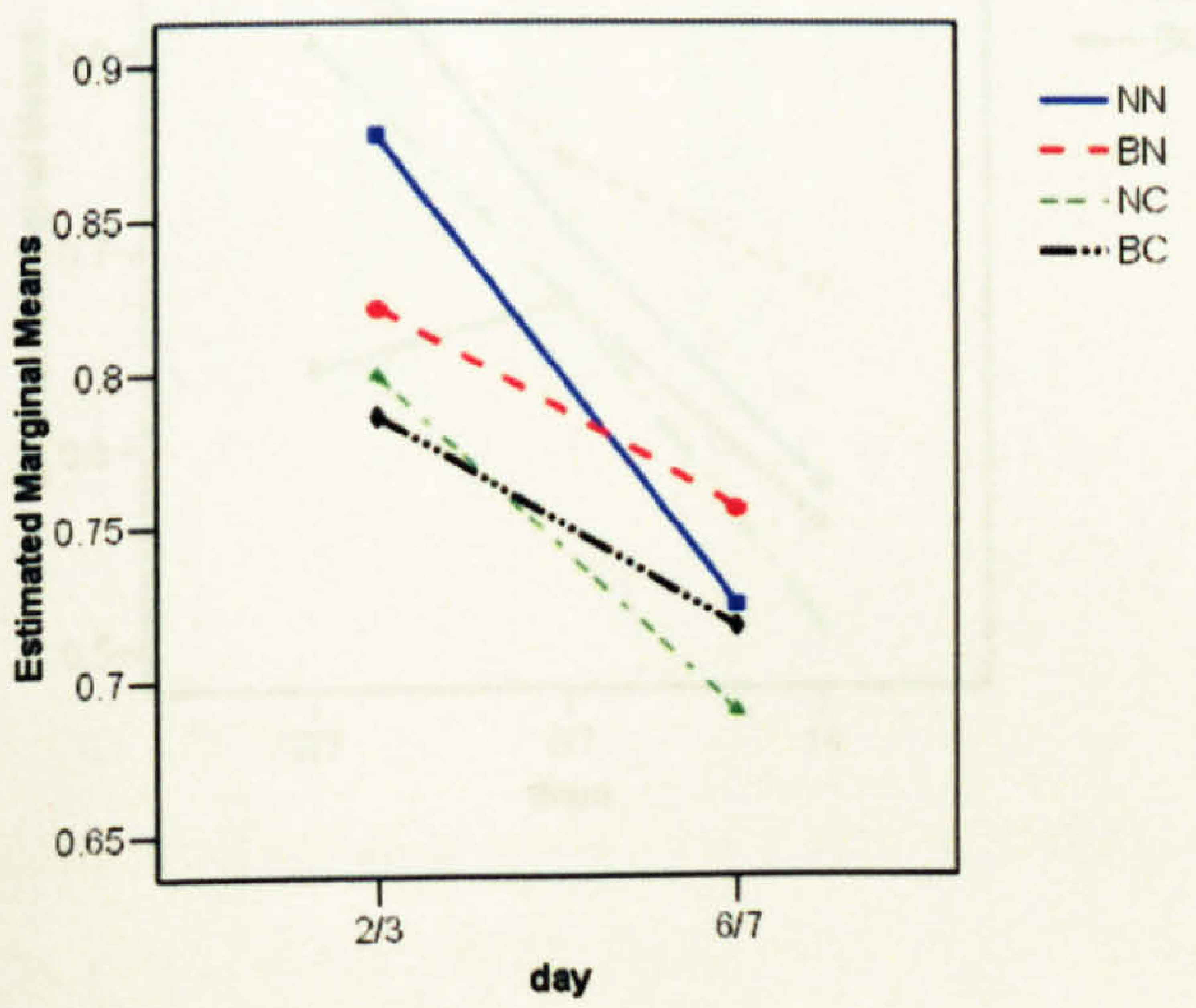
Transformed CC d2/3, d6/7 (day) = box|contact + sex|ent + stray + age_{covar} (N = 69)

Sex|ent, stray and age had no effect, so they were removed from the equation:.

Transformed CC d23, 67 = Box|contact (N = 69)

Multivariate tests were all NS at $p > 0.1$ apart from day ($p < .001$). Within-subjects effects were identical. All between-subjects effects were NS at $p > 0.1$. There was little difference between Nbox and Box (estimated marginal means of Nbox = 6.93, of Box = 6.89). There was more difference between Ncontact and Contact (estimated marginal means: Ncontact 7.24, Contact 6.60). Marginal means of CC by day, split by treatment group are in Figure 3.7.

Figure 3.7 Estimated marginal means for log transformed CC by day, split by treatment group. GLM equation: Transformed CC d23, 67 = Box|Contact (N = 69)



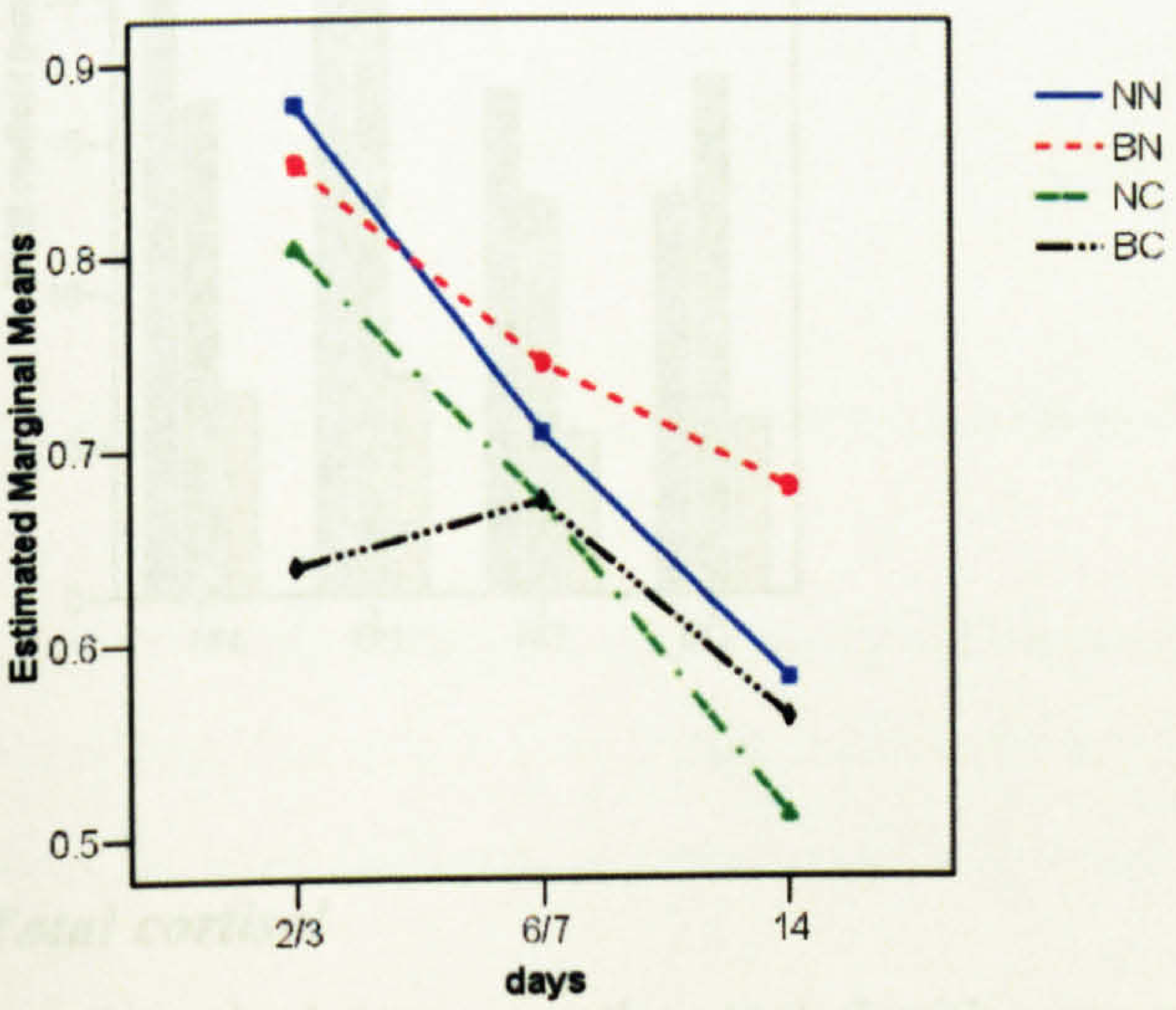
Adding day 14 data as a dependant (y) variable into the equation reduced N to 38. Starting off with the same long equation as above, the only significant terms were box and contact, so the GLM was re-run with:

Transformed CC d23, 67, 14 = Box|Contact (N = 38)

Multivariate tests were all NS at $p > 0.1$ except for day ($p < .001$) and day*box ($p = .046$). Within-subjects effects were all NS at $p > 0.1$ apart from day ($p = .000$) and box ($p = .019$). Between-subjects effects were all NS at $p > 0.1$ apart from Contact ($p = .047$), with the contact group having lower CC (estimated marginal means after back transformation: Ncontact = 6.50; Contact = 5.40). There was no significant difference between Box groups (estimated marginal means after back transformation: Nbox = 5.92, Box = 5.91).

Removal of the box*con term causes the between-measures effect of contact to become NS at $p > .191$, though the difference between marginal means for Ncontact and Contact remain similar. This difference appears to be caused by BC being low on day 2/3 (Fig. 3.8).

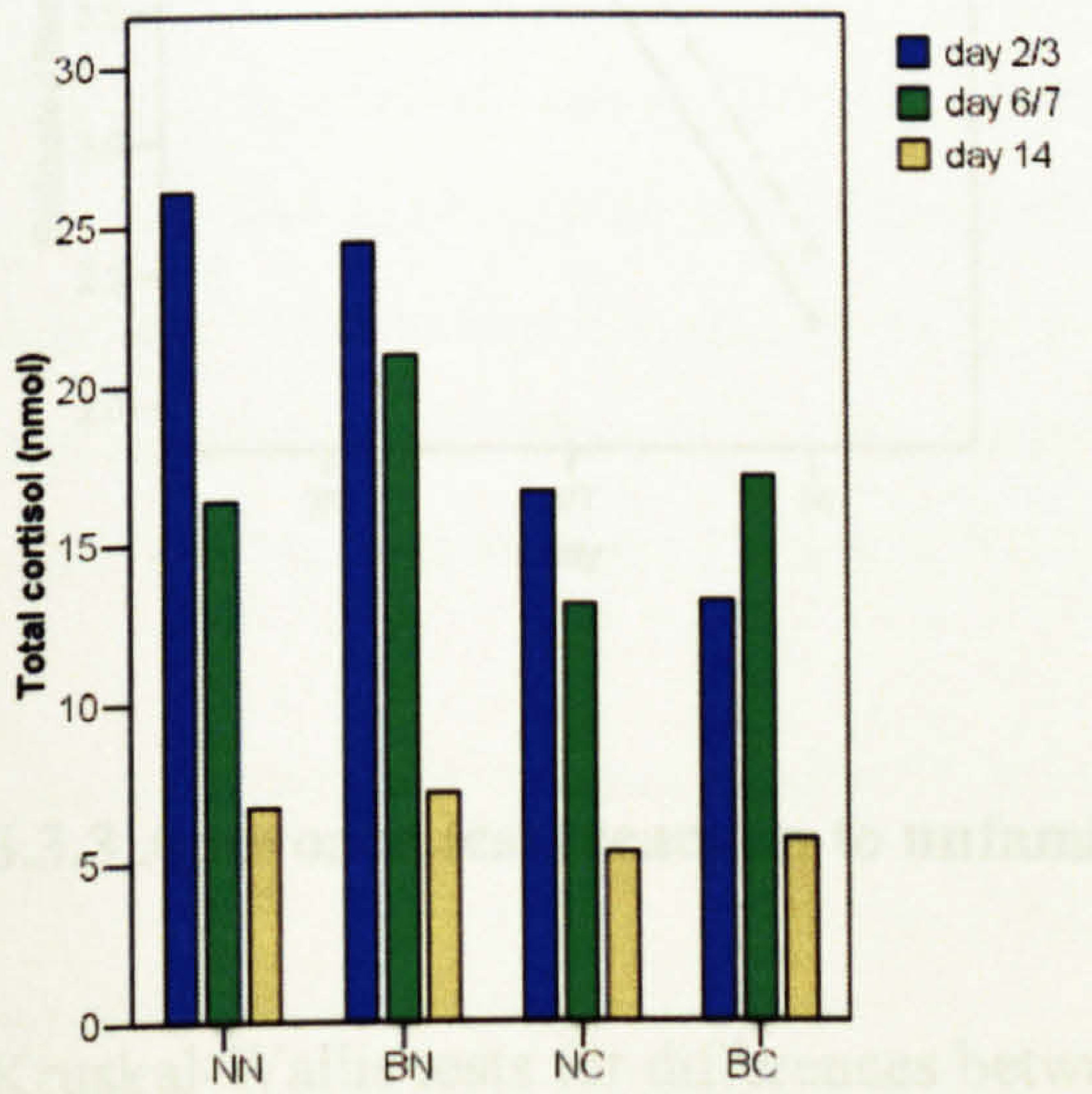
Figure 3.8 Estimated marginal means for log transformed CC by day, split by treatment group. GLM equation: Transformed CC d23, 67, 14 = Box|Contact (N = 38)



The CC ratio fell over time, indicating that cats became less physiologically stressed over the first fortnight. Over days 2/3 and 6/7, none of the treatment groups had a significant effect on CC. Adding day 14, contact has a significant between-subjects effect at $p = .047$, with Contact cats having lower CC ratios than Ncontact cats. However, since removing the non-significant box*con term renders the Contact term NS, it is ultimately a non-significant between-subjects effect caused by low CC on day2/3 in the BC group (Fig. 3.9). It does not appear in the previous analysis and is due to several of the BC cats with high CC on day2/3 having no day 14 data (rehomed, or still remaining in admissions due to lack of space). The within-cat differences caused by the box treatment is also due to this low d2/3 value for the BS group. In conclusion, there was no reliable evidence for a significant effect of any of the treatment groups on CC.

Other than a significant drop over time, no terms in the repeated measures equation had a significant effect on CC d2/3, d6/7, nor when d14 was added to the equation. The total cortisol produced by day, split by treatment group is in Figure 3.9.

Figure 3.9 Total cortisol by day, split by treatment group



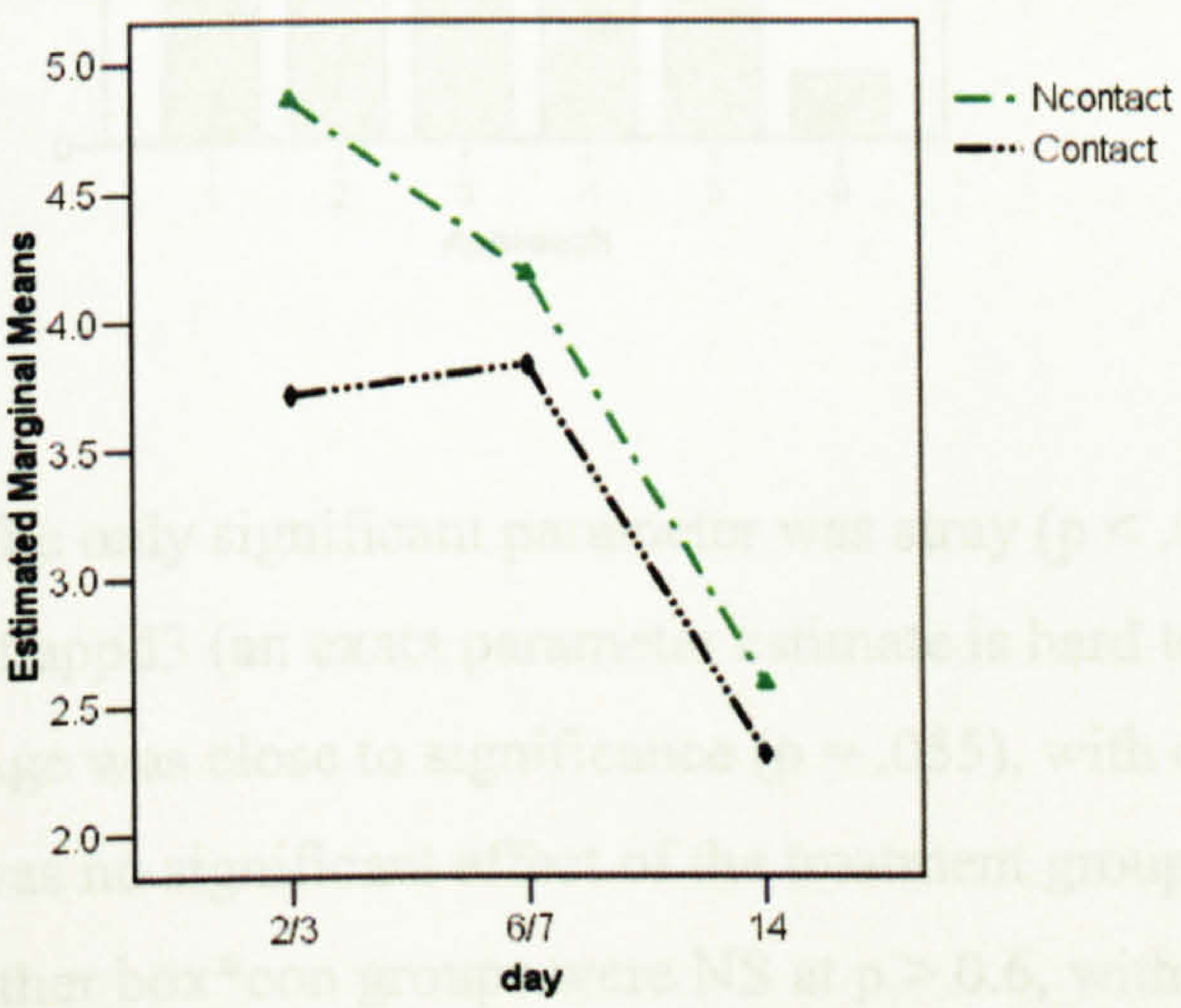
Total cortisol

Total cortisol data were then tested with a repeated measures GLM with the same factors as above. With day2/3 and day6/7 in the equation (N=66), the only significant effect within cats was a drop over time, and no terms caused significant differences between cats. Adding day 14 data to the equation reduced N to 31. The best fitting equation was:

Transformed total cortisol d2/3, 6/7, 14 = box|contact

There was a significant overall decrease over time (within-subjects effect of day p = .000), and a between-subject effect of the Contact treatment group, with Contact cats lower (p = .030). The effect of Contact on total cortisol is again only due to the removal of high total cortisol day 2/3 BC cats (Figure 3.10) and should be ignored. Removing the non-significant box*contact term reduced the p-value of the between - subjects Contact term to p = .024, so Contact was not significantly lower due to a chance interaction with Box.

Figure 3.10 Estimated marginal means for transformed total cortisol by day, split by Contact treatment group.



3.3.3 Approach test (reaction to unfamiliar female person)

Kruskal-Wallis tests for differences between all four treatment groups were NS for every test day at p > 0.1. Differences between Box and Nbox groups were NS for all 3 days, as were differences between Contact and NContact groups.

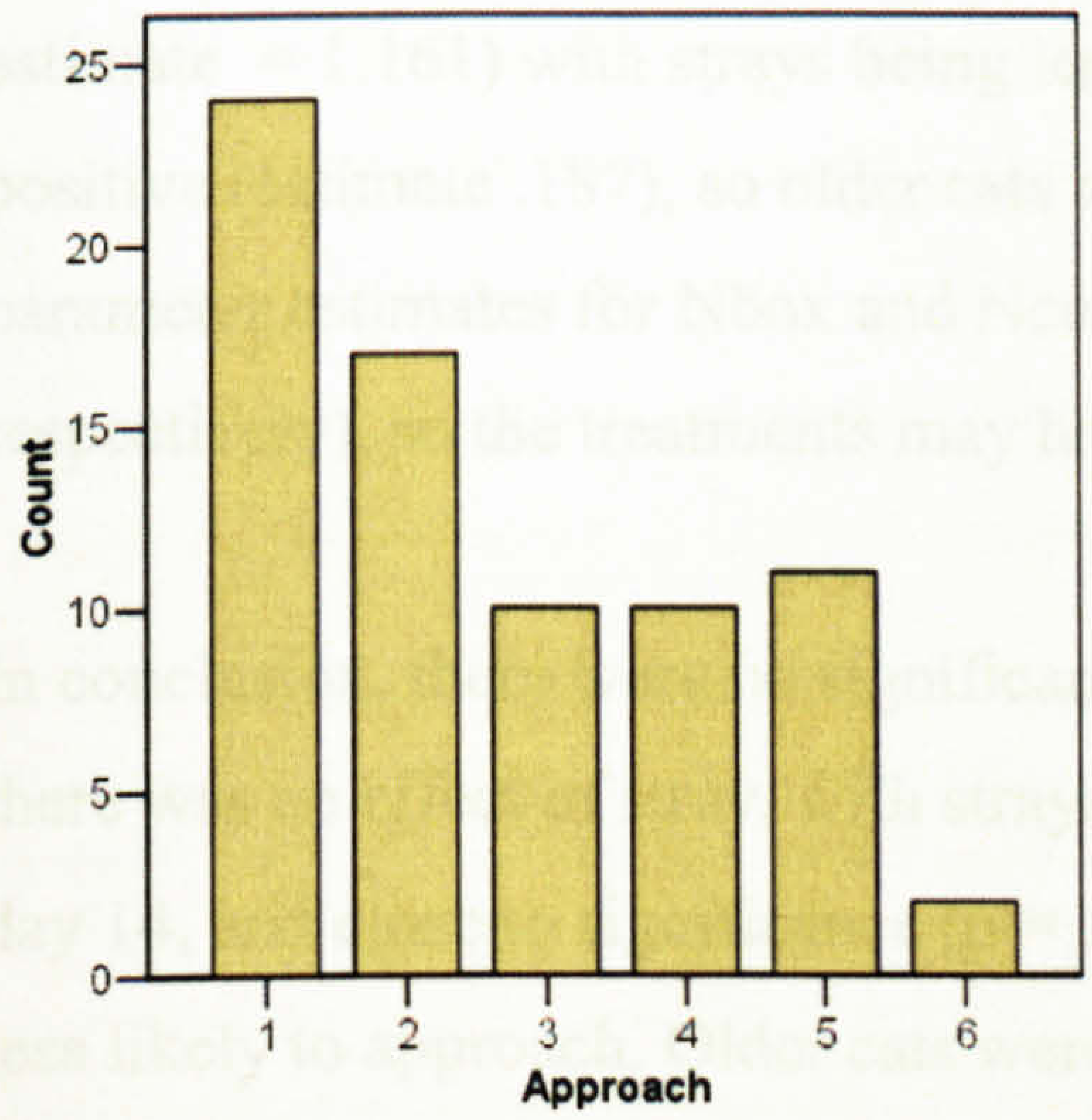
Approach data from day 3 (Fig. 3.11) was analysed with the starting equation:

Appd3 = box|con + fem|ent + stray + age

The best fitting equation was:

Appd3 = Box|con + stray + age

Figure 3.11 Numbers of cats for each of the approach scores, day 3.



The only significant parameter was stray ($p < .004$), with strays having higher values of appd3 (an exact parameter estimate is hard to determine with ordinal regression). Age was close to significance ($p = .055$), with older cats having higher scores. There was no significant effect of the treatment groups, though box*con NN had $p = .064$ other box*con groups were NS at $p > 0.6$, with NN higher than the other groups.

The same was carried out for day 7, and the best fitting equation was:

Appd7 = Box + stray + age

The model fit was significant at $p = .020$ with stray as the only significant parameter ($p = .011$). Again, strays tended to have higher approach scores. Box ($p = .112$) and age ($p = .105$) were the other two parameters with highest F-values. (Older cats possibly having higher categories, and cats with boxes possibly scoring higher).

For approach on day 14, the best equation was:

Appd14 = Box + con + stray + age

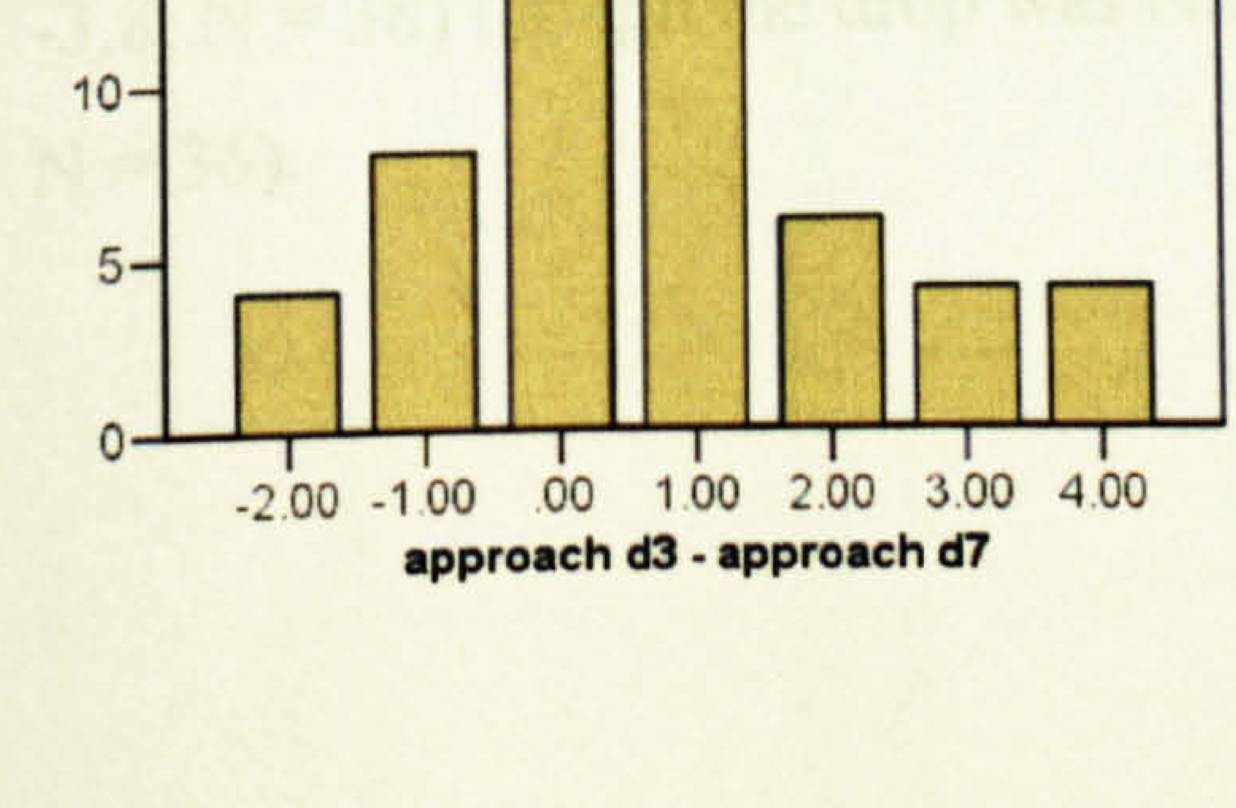
The model fit however was not significant ($p = .057$), and the pseudo R-Sq was low, at .209. Stray ($p = .019$) and age ($p = .037$) were the only two parameters with significant p-values, though Contact and Box were close with $p = .051$ and $p = .070$

respectively. The effect of stray made up a large part of the R-sq value (parameter estimate = 1.161) with strays being less likely to approach. The effect of age was positive (estimate .187), so older cats were generally less likely to approach. The parameter estimates for Nbox and Ncontact were positive (.825 and .949 respectively), so the treatments may have increased the chance of an early approach.

In conclusion, there were no significant treatment effects on days 3, 7 or 14, though there was an effect of stray, with strays having higher scores. Age was significant on day 14, and close to significance ($p = .055$) on day 3, both times with older cats being less likely to approach. Older cats were also less likely to approach on day 7, though this was NS at $p = .105$.

Approach day 3 – *approach* day 7 is in Figure 3.12. Although a score of zero (no change) was the single highest score, more cats scored positively than negatively, i.e. more became more likely to approach over time, and approach sooner. This difference between days 3 and 7 was significant (Wilcoxon signed ranks, $p = .001$). There was no significant difference between days 7 and 14 (Wilcoxon signed ranks, $p = .815$).

Figure 3.12 Approach d3 – approach d7. Positive ranks indicate a fall (becoming more likely to approach) over that time.



The Kruskal Wallis test between all 4 treatment groups was significant at $p = .016$ and suggested that cats without boxes were more likely to have a greater decrease in approach between days 3 and 7 (Table 3.8).

A Mann-Whitney test between Box and Nbox cats was significant at $p = .003$, with Nbox having a higher mean rank. There was no significant difference between Ncontact and Contact (Mann-Whitney test NS at $p > 0.1$).

Table 3.8 Table of ranks, from Kruskal-Wallis test of appd3 – appd7. Since the data approximate a normal distribution, absolute means for each treatment group are also given.

Ranks

		N	Mean Rank	Mean value
app3rec - app7rec	NN	19	47.9	1.37
	BN	17	28.3	0.00
	NC	19	41.3	0.74
	BC	19	31.6	0.21
	Total	74		

From the Kruskal-Wallis test on *approach*3, the mean rank of Nbox was higher than the mean rank of Box, though NS at $p > 0.1$ (Mann-Whitney, $p = .135$; Fig. 3.13a). This suggests that the significant drop could be due to Nbox cats starting off with a higher approach score on day 3. However, the mean rank of Nbox was lower on day 7 than the mean rank of box, though NS at $p > 0.1$ (Mann-Whitney $p = .153$; Fig 3.13b). Day 14 values are not significantly different between box and Nbox either (Mann-Whitney $p > 0.2$; Fig 3.13c).

Examining the change over time for Nbox cats and Box cats separately, the drop between day 3 and day 7 was significant for the Nbox group (Wilcoxon, $p < .001$, $Z = -3.8$, $N = 38$) though the drop was NS for the Box group (Wilcoxon $p = .665$, $Z = -.43$, $N = 36$).

Figure 3.13a The number of cats exhibiting each class of approach behaviour on day 3, split by the Box condition

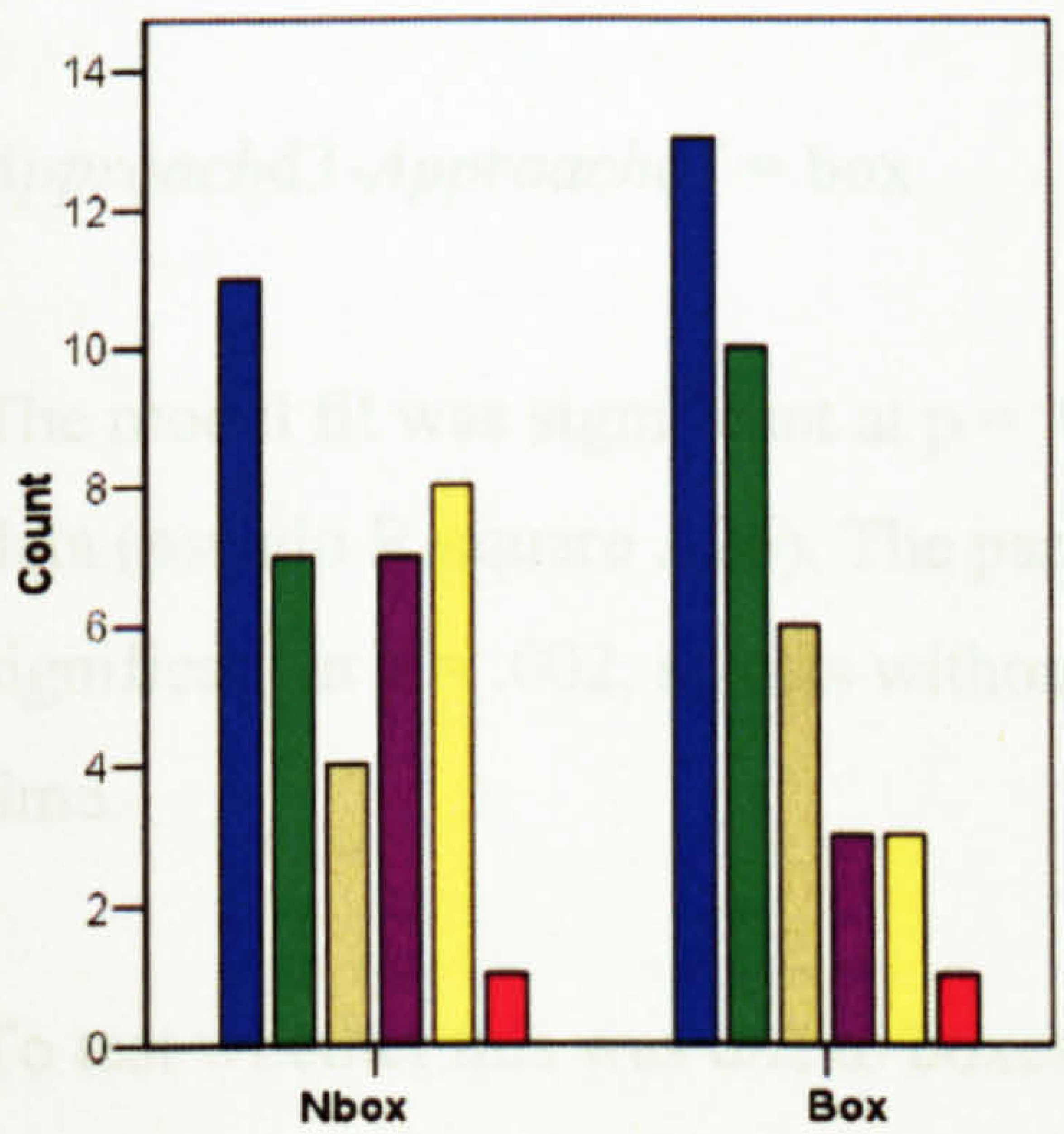


Figure 3.13b The number of cats exhibiting each class of *approach* behaviour on day 7, split by the Box condition

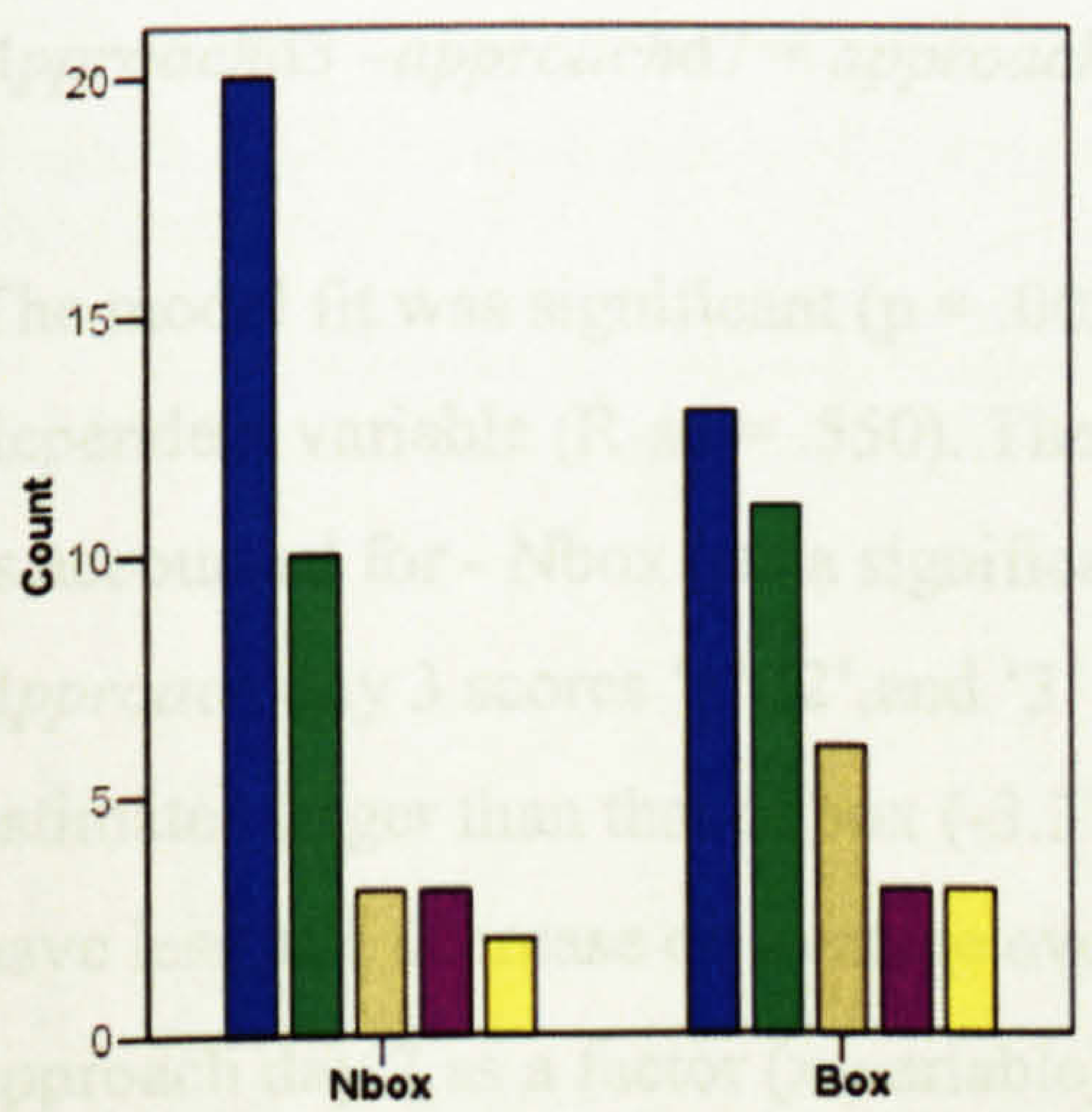
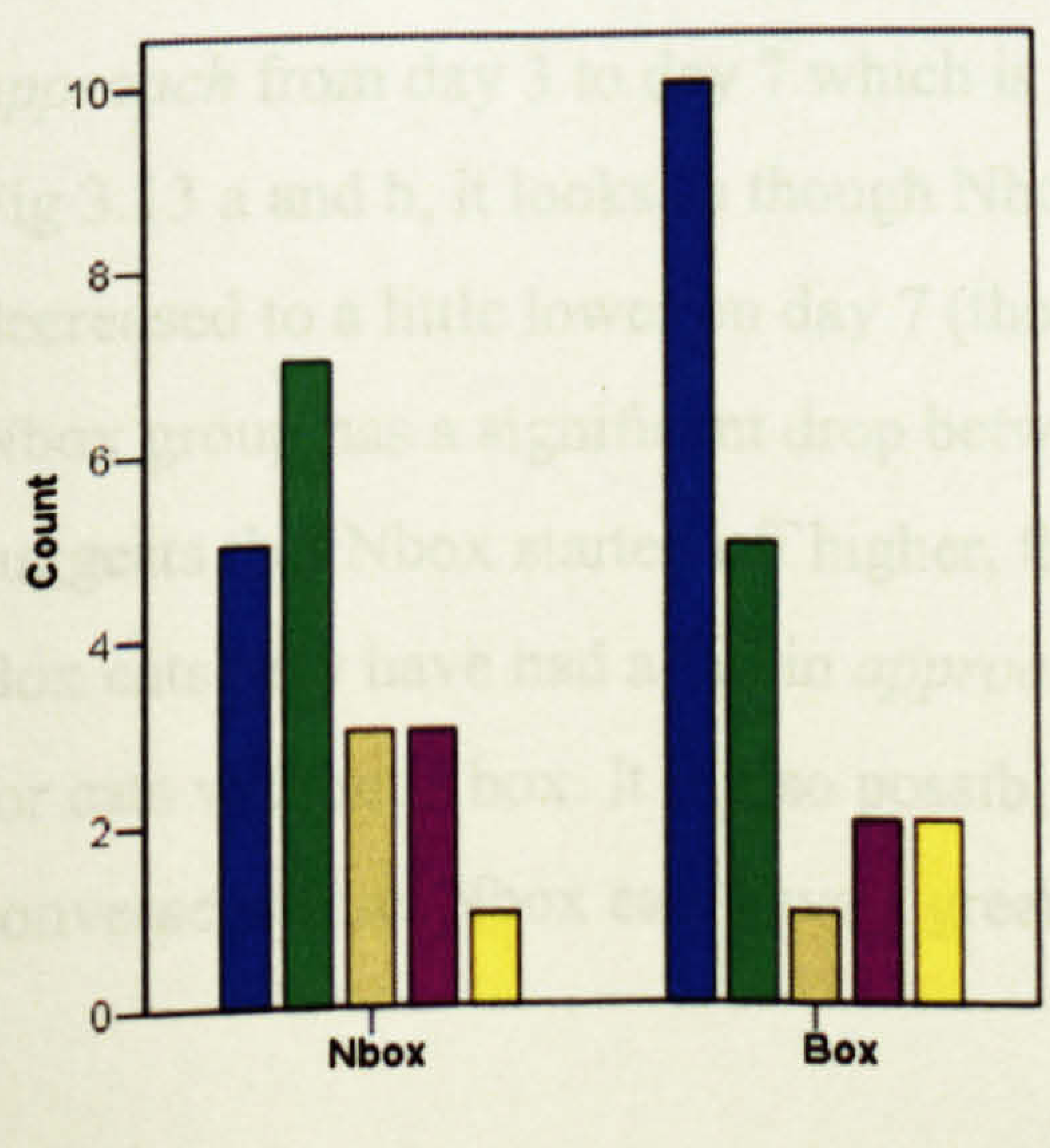


Figure 3.13c The number of cats exhibiting each class of *approach* behaviour on day 14, split by the Box condition



The only variable of the ordinal regression that significantly contributed to the model was box:

*Approach*d3-*Approach*d7 = box

The model fit was significant at $p = .002$, though explained little of the variation in the data (pseudo R-square .126). The parameter estimate for Box was positive, and significant at $p = .002$, so cats without a box are more likely to have a decrease over time.

To test whether this was due to boxes making cats less likely to approach, or due to boxes lowering approach scores on day 3, an ordinal regression was performed which factored out day 3 scores:

*Approach*d3 –*approach*d7 = *approach*d3 + Box

The model fit was significant ($p = .002$), and was reasonable at explaining the dependent variable (R-sq = .550). There is still an effect of box once *approach* day 3 is accounted for - Nbox has a significant ($p = .010$) positive parameter estimate (.690). *Approach* day 3 scores '1','2',and '3' all had significant ($p < 0.01$) parameter estimates larger than that of box (-3.31, -2.27, -3.02). As above, cats with boxes will have less of a decrease on average over days 3 to 7. Replacing approach day 3 with approach day 7 as a factor (x-variable) and repeating gives similar p-values and estimates.

So, Nbox cats are more likely to have a decrease, and have a greater decrease, in *approach* from day 3 to day 7 which is not due to having high scores on day 3. From Fig 3.13 a and b, it looks as though Nbox was much higher than Box on day 3, then decreased to a little lower on day 7 (though differences between groups were NS). Nbox group has a significant drop between days 3 and 7, and box does not, which suggests that Nbox started off higher, then decreased by day 7. This suggests that the Box cats may have had a fall in *approach* before day 3, but that this fall was delayed for cats without a box. It is also possible (though less likely from the data) that the converse is true. Nbox cats have a greater proportion of cats in *approach* class '1' on day 14, but less in class '2' (Fig. 3.13c) which may suggest that boxes encourage long-stay cats to remain inside until the door opens, though given the smaller sample size on day 14, this is speculative. So, any difference caused by Box acts on the rate of decrease, not the final level *per se*.

3.3.4 Social (reaction to familiar male person)

Firstapp

There is little observable difference between treatment groups in the social test *firstapp* (Fig. 3.14), though NN is a little higher on days 3 and 4. Most cats do not approach the observer (score 3 or higher, Fig. 3.15), though around a third (score 4 or higher) appear to find the test aversive.

Figure 3.14 The median class of *firstapp* shown by cats each day over the first week, split by treatment group

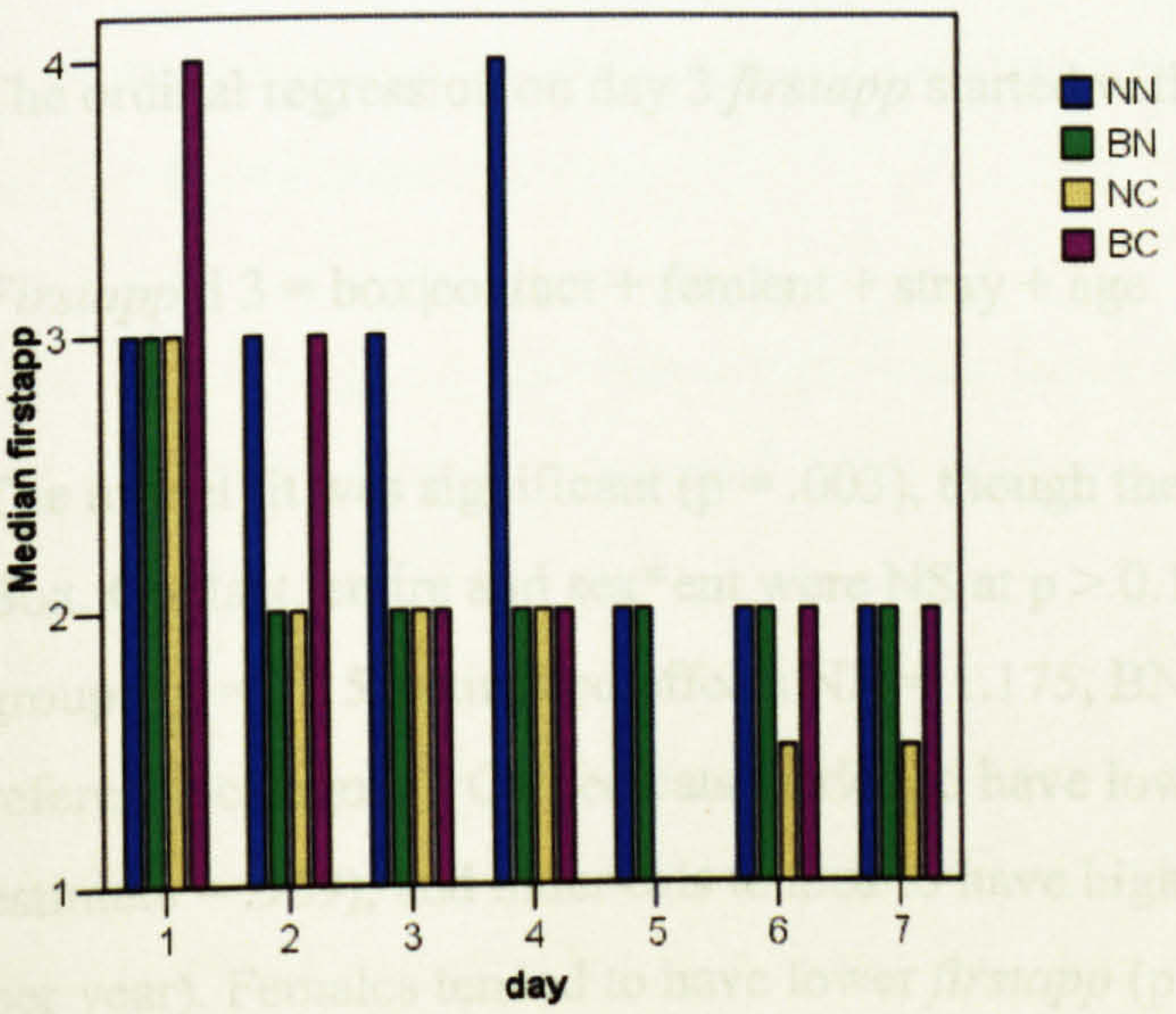
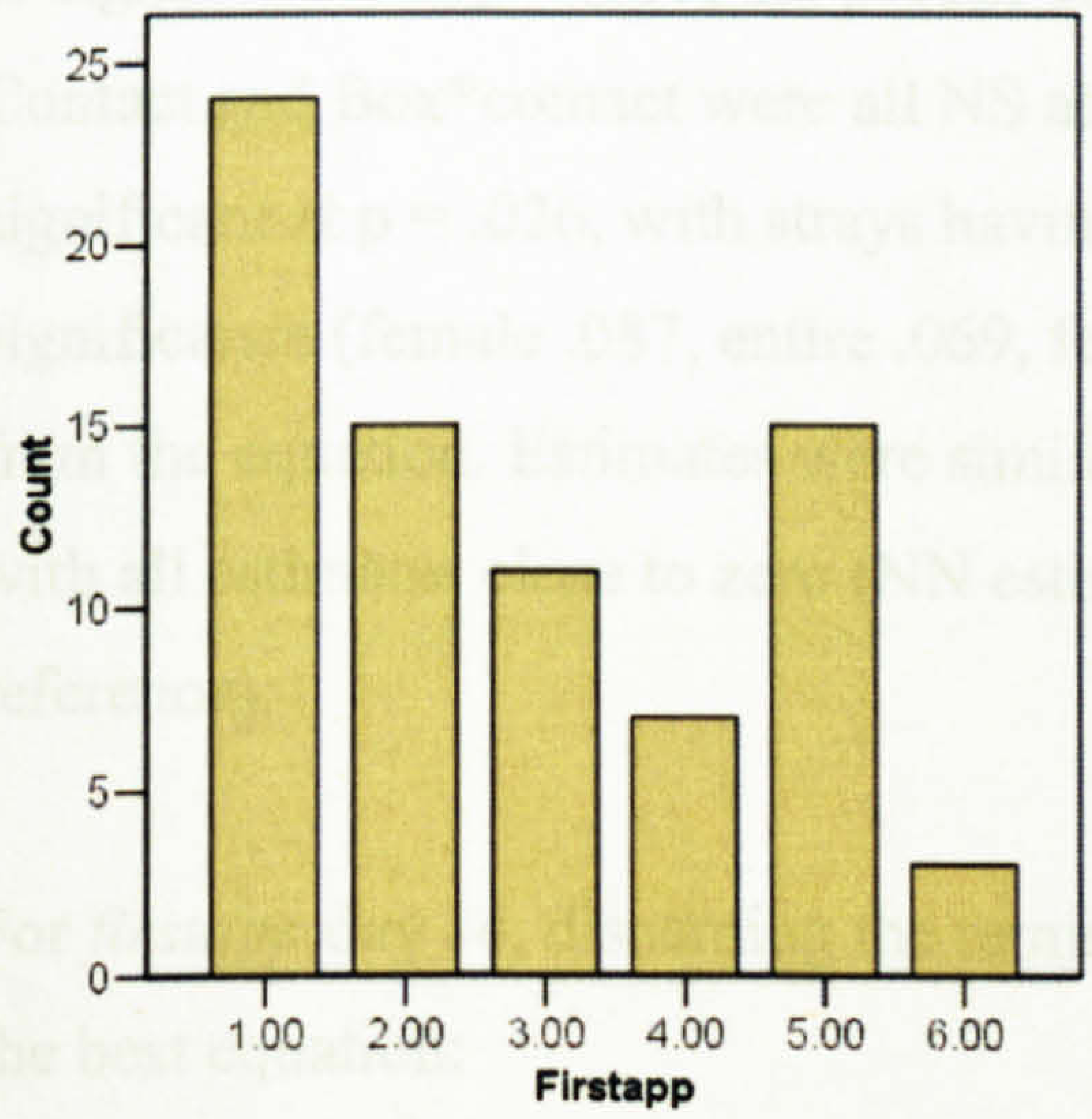


Figure 3.15 The number of cats exhibiting each class of *firstapp* on day 3 (data from all cats)



The Kruskal-Wallis tests for differences between treatments on each day were NS at $p > 0.1$, as were Mann-Whitney tests on each day for differences between Nbox and Box, and Ncontact and Contact.

The ordinal regression on day 3 *firstapp* started with the initial equation:

Firstapp d 3 = box|contact + fem|ent + stray + age

The model fit was significant ($p = .003$), though the pseudo R-sq was low, at .274. Box, Contact, entire and sex*ent were NS at $p > 0.1$. NN was higher than the other groups ($p = .015$, estimated effects NN = 1.175, BN = -.332, NC = -.019, BC was the reference category). Owned cats tended to have lower *firstapp* (stray $p = .007$, estimate = .989), and older cats tended to have higher *firstapp* ($p < .001$, estimate .280 per year). Females tended to have lower *firstapp* ($p = .044$, estimate = -1.44).

Sex*ent and entire were retained to keep the equation the same as day 7 (below). When sex*ent and entire were removed, sex became NS at $p = .076$. When sex was also removed, the parameter estimates and p-values for the remaining variables were virtually unchanged.

For *firstapp* day 7, with the same starting equation as above, the model fit was close to significance at p = .085, the pseudo R-sq was.175. Parameter estimates for Box, Contact and Box*contact were all NS at p > 0.7. Of the other factors, stray was significant at p = .026, with strays having higher *firstapp*. The rest were close to significance (female .087, entire .069, fem*ent .051, age .058) so were not removed from the equation. Estimates were similar to day 3 for all factors except box*con, with all estimates close to zero (NN estimate = -.115, BN = -.155, NC = .101, BC reference).

For *firstapp* day 14, discarding the terms with no significant effect on the model left the best equation:

App day 14 = box|contact

The model fit was NS at p > 0.1, and the pseudo R-sq was low at .087, with parameter estimates all NS at box = .120, contact = .094 and box*contact = .191. Removing box*contact left box and contact both NS at p > 0.4. Although non significant, parameter estimates for stray and age have the same sign as *firstapp* days 3 and 7 (.308 and .041 respectively).

Thus treatment group was only a significant factor on day 3, with NN being less likely than the other 3 groups to approach. This effect might be present only when the cats are still acutely stressed.

The effects of variables upon approach tests were similar for days 3 and 7: strays and old cats are less likely to approach, the same as the staff approach test. Though sex, entire and the interaction between them were close to significance on days 3 and 7 (though not on day 14), overall they were non-significant. On both days 3 and 7, entire females were most likely to approach: on day 7 the other 3 groups were all roughly equally less likely; on day 3 male, entire were least likely to approach, with both male and female neuters in-between.

Social 1st minute

There was no observable difference between treatment groups in *soc1st* (Fig. 3.16). Most cats were friendly (Fig. 3.17).

Figure 3.16 The median class of *soc1st* shown by cats each day over the first week, split by treatment group

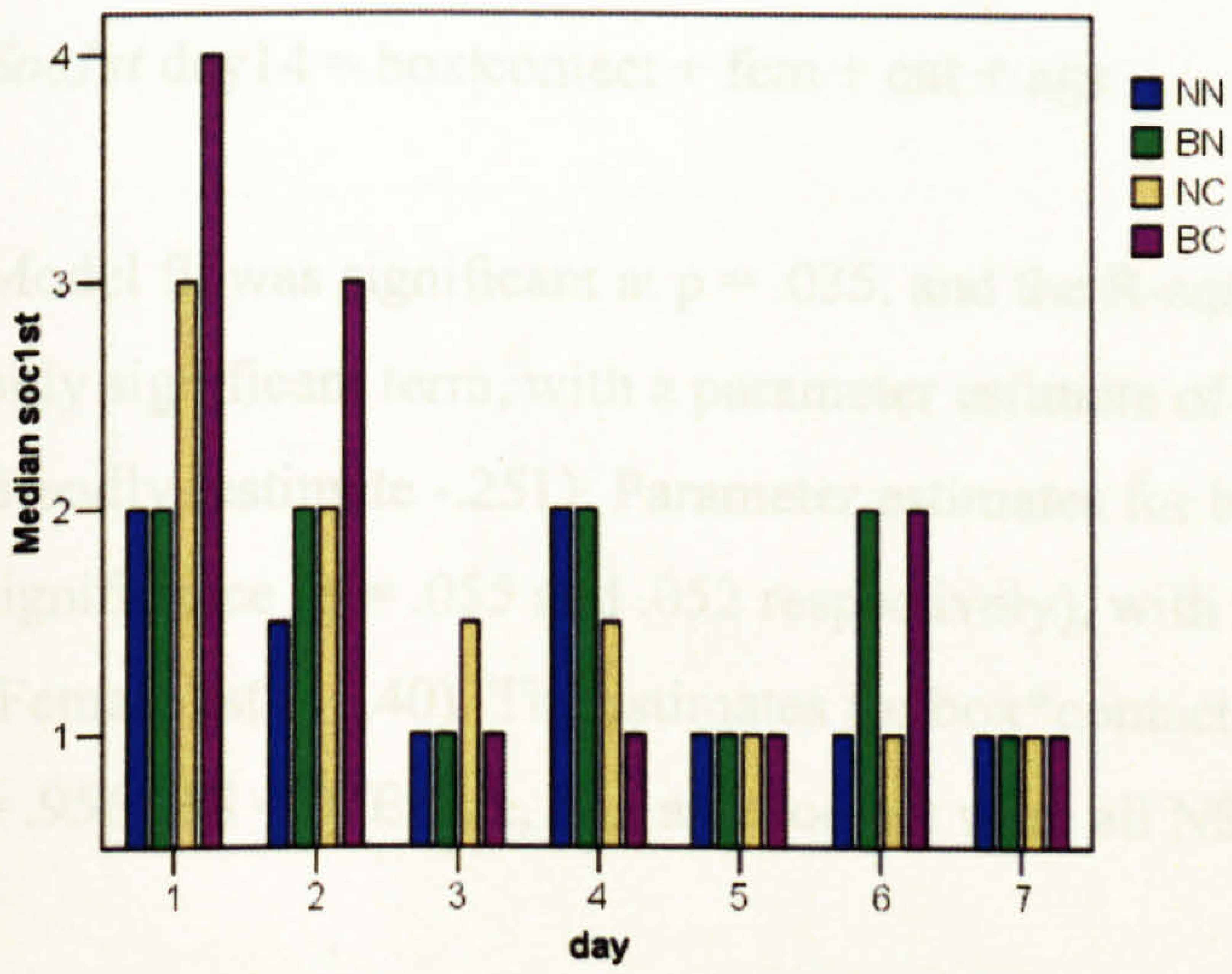
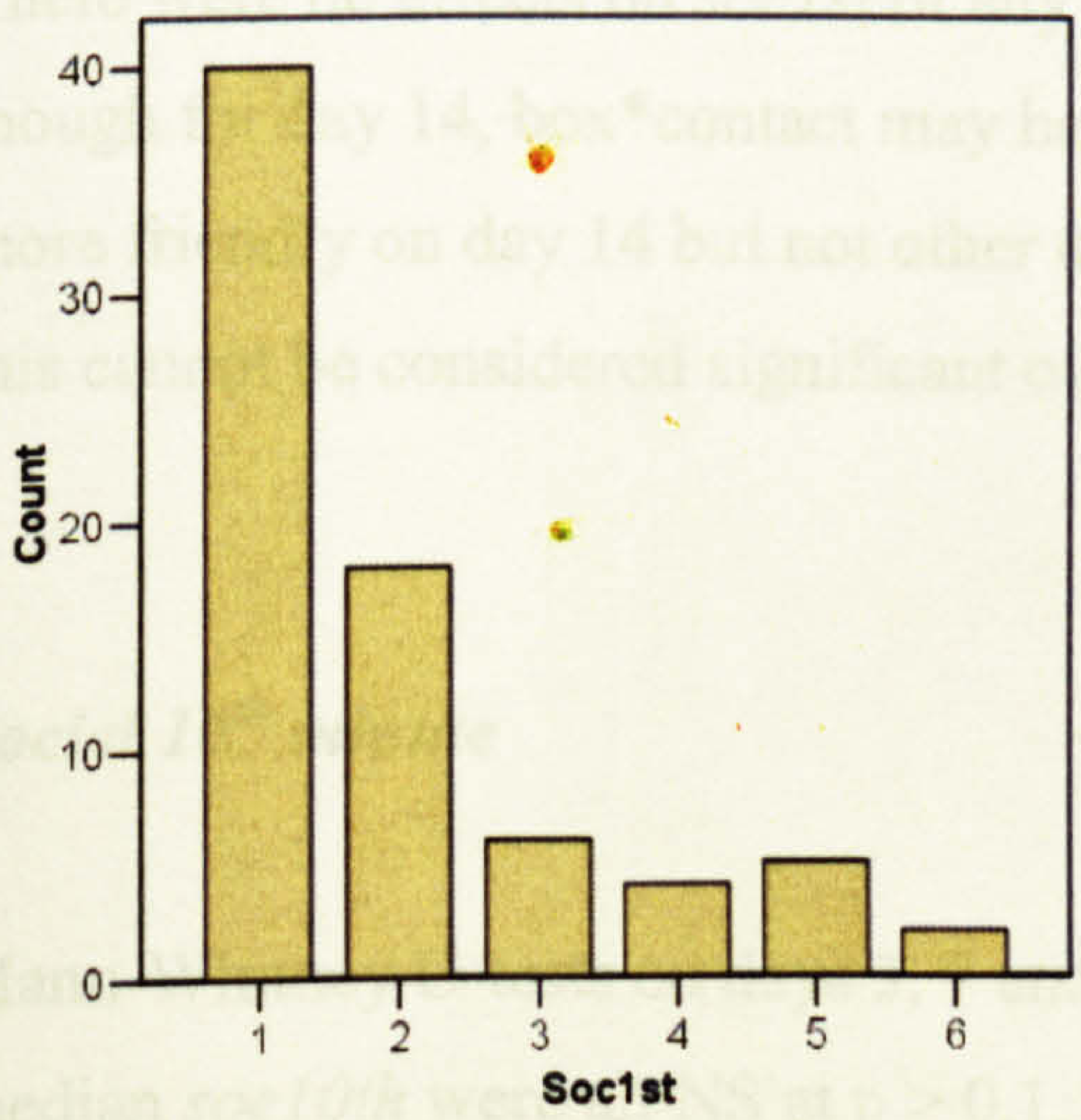


Figure 3.17 The number of cats exhibiting each class of *soc1st* on day 3 (a typical day, data from all cats)



Kruskal-Wallis tests between the four treatments for each day were all NS at $p > 0.1$ for days 1-7 and 14. Mann-Whitney tests on Box and Nbox, and on Contact and Ncontact were also all NS at $p > 0.1$.

For ordinal regression on day 3, with the standard initial equation, model fit was NS at $p = .851$ and the R-square was low at .055. All parameters were NS at $p > 0.2$, and did not improve when variables that contributed least to the model were removed. Similar results were found for *soc1st* day 7.

For Soc1st day 14, the best model was

Soc1st day14 = box|contact + fem + ent + age

Model fit was significant at $p = .035$, and the R-square was low at .328. Age was the only significant term, with a parameter estimate of $p = .042$, older cats being more friendly (estimate -.251). Parameter estimates for box*contact, sex, were close to significance ($p = .055$ and .052 respectively), with females being less friendly (Female est = 1.40). The estimates for box*contact were NN = -2.02, BN = -.114, NS = .956, BS = 0. Entire, box and contact were all NS at $p > 0.1$.

Removing the box*contact term leaves box NS at $p > 0.7$, and contact close to significance at $p = .080$, with a parameter of contact = 1.242.

There were no effects on *soc1st* of any of the treatment groups on days 3, 7 or 14, though for day 14, box*contact may have had an effect ($p = .055$). Older cats were more friendly on day 14 but not other days. Given the relatively high p-value of .042, this cannot be considered significant overall.

Social 10th minute

Mann-Whitney U-tests on days 3, 7 and 14 for median *approach*, median *soc1st* and median *soc10th* were all NS at $p > 0.1$, so there was no effect of box on friendliness in the 10th minute of the social test.

Changes over time

Most cats became more likely to approach over time (Fig. 3.18) and became more friendly over days 3 to 7 (Fig. 3.19). There was a significant decrease between days 3 and 7 for both *firstapp* (Wilcoxon signed ranks, $p < .001$) and *soc1st* (Wilcoxon signed ranks, $p = .003$). There was no significant change between days 7 and 14 for either variable (Wilcoxon signed ranks, $p > 0.1$).

Figure 3.18 The number of cats exhibiting each class of *appchange* (data from all cats)

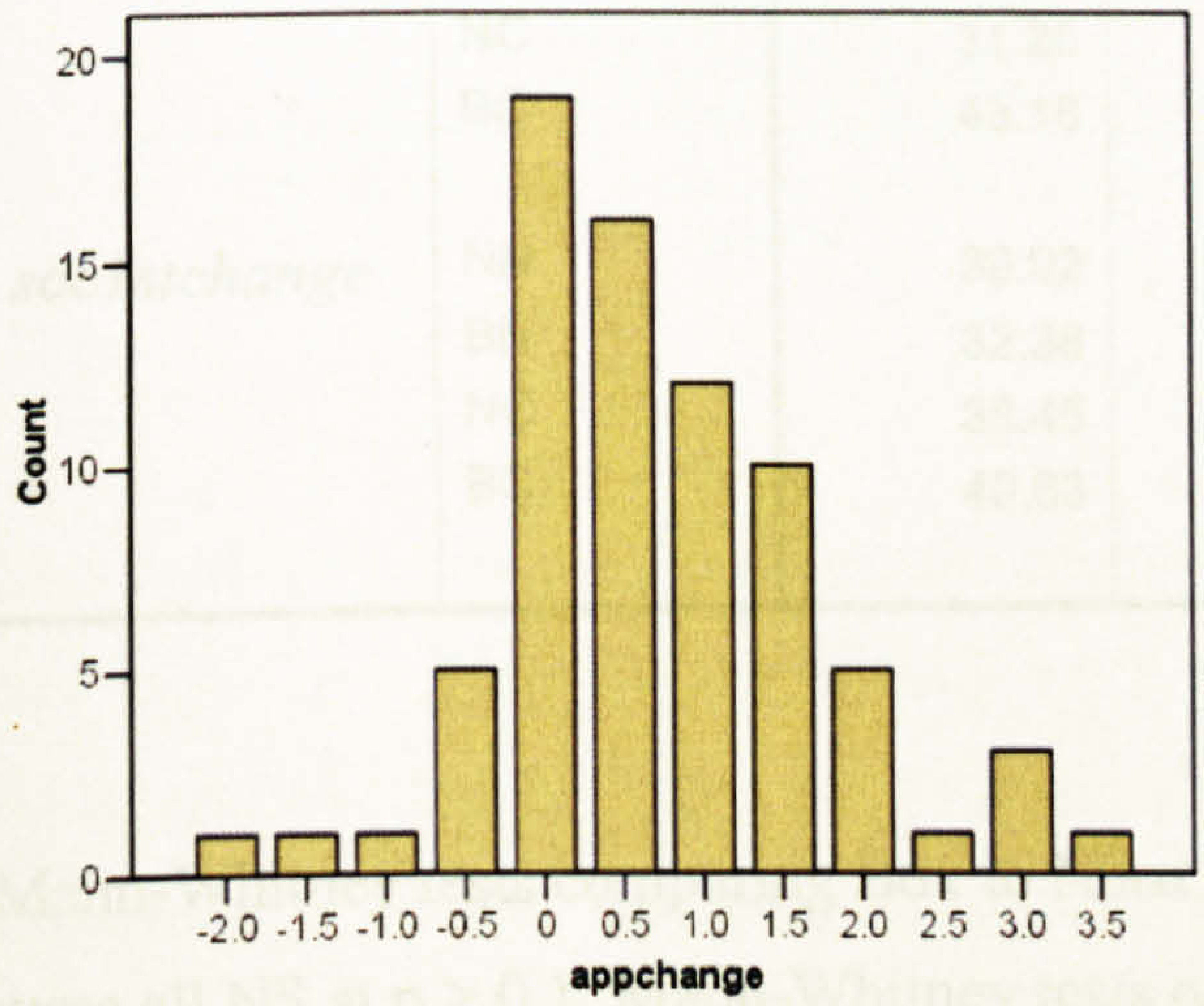
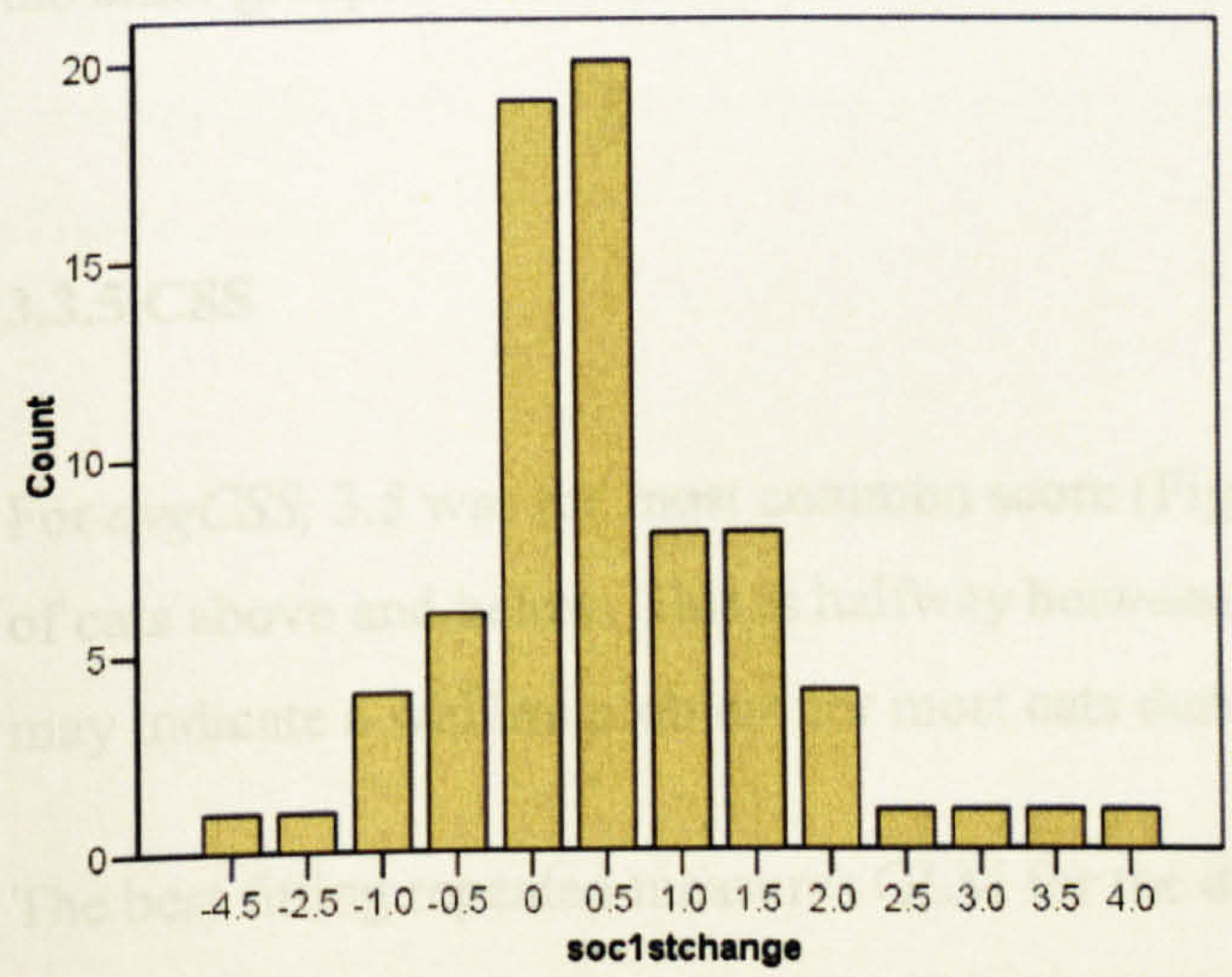


Figure 3.19 The number of cats exhibiting each class of *soc1stchange* (note the scale change to the left of the origin, data from all cats)



A Kruskal-Wallis test between treatments on *appchange* was significant at p = .028, with the BN and NC groups lower than NN and BC. *Soc1stchange* was NS at p > 0.1 (Table 3.9).

Table 3.9 Table of ranks from Kruskal-Wallis tests on *appchange*, and *soc1stchange*. Since the data approximate a normal distribution, absolute means for each treatment group are also given.

	boxcontact	Mean Rank	Mean value
appchange	NN	47.26	1.05
	BN	29.79	0.353
	NC	31.25	0.375
	BC	43.18	1.03
soc1stchange	NN	39.92	0.711
	BN	32.38	0.118
	NC	38.45	0.525
	BC	40.63	0.579

Mann-Whitney tests comparing Box to Nbox, and comparing Contact to Ncontact, were all NS at p > 0.1. Mann-Whitney tests on each possible pair of treatment groups were all NS at p > 0.1 apart from NN v BN, p = .023, NN v NC, p = .008 and BN v BC, p = .093. Since NN had higher *firstapp* on day 3, this would have allowed a greater drop, though this is not true for BC, which was not significantly different from the other groups.

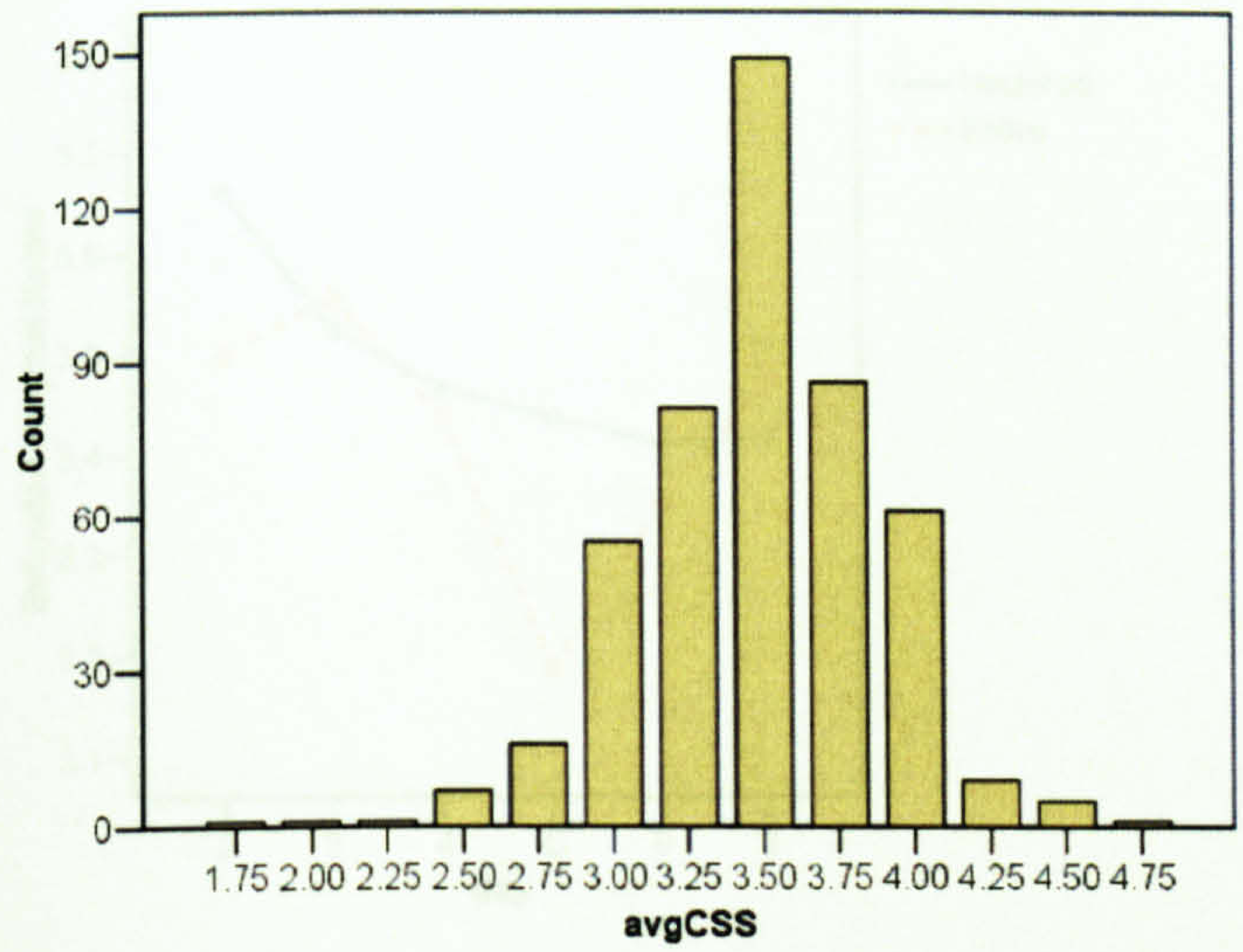
3.3.5 CSS

For *avgCSS,* 3.5 was the most common score (Fig. 3.20) with roughly equal numbers of cats above and below. This is halfway between weakly tense and very tense, and may indicate a welfare problem for most cats during the week after admission.

The best fitting repeated measures GLM for the dependant variable '*avgCSS* days 2,3,4,5,6,7' was:

*avgCSS*days 2,3,4,5,6,7 = box|contact + entire + age

Figure 3.20 Histogram of *avgCSS* (all cats, days 1-7)



Multivariate tests were significant for day ($p = .002$), day*box ($p = .080$) and day*entire ($p = .020$), the rest NS at $p > 0.1$. Within subjects effects were NS at $p > 0.1$ apart from day ($p = .000$) and day*entire ($p = .049$), with *avgCSS* generally decreasing over time. Neutered cats showed a smooth curve over time, while entire cats show a drop to day 4, then climb back up to day 6 (Fig. 3.21).

Between subjects effects were significant for box ($p = .005$) and age ($p = .042$), the rest were NS at $p > 0.1$. The Box groups had lower *avgCSS* than the Nbox group (Figure 3.22), and the effect of age varied nonsystematically across days from between .003 to .040. There was no effect of Contact (Figure 3.23), or box*contact (Figure 3.24).

Figure 3.21 Estimated marginal means for *avgCSS*, split by neuter status.

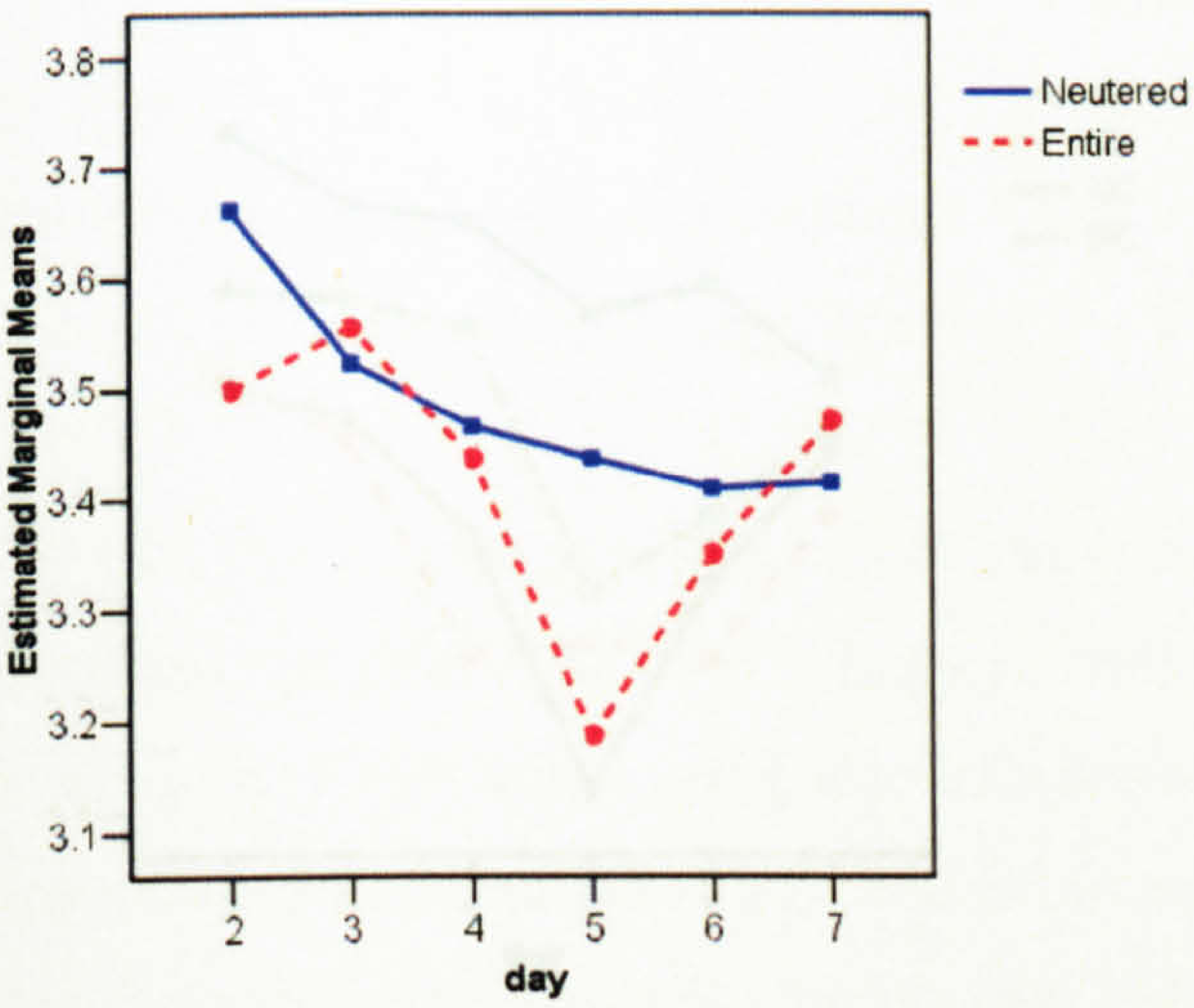


Figure 3.22 Estimated marginal means for CSS, split by the box treatment

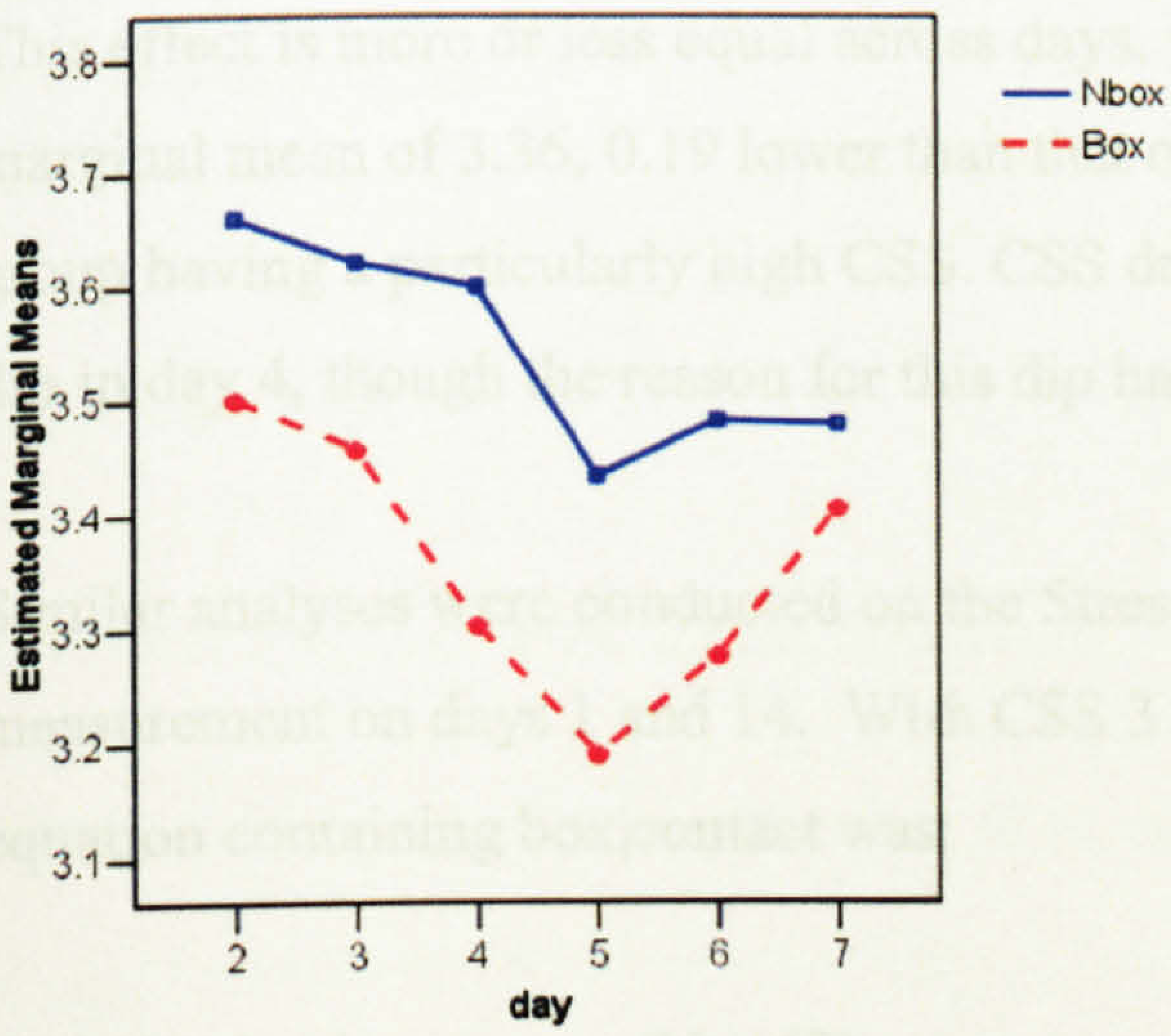


Figure 3.23 Estimated marginal means for CSS, split by the social contact treatment.

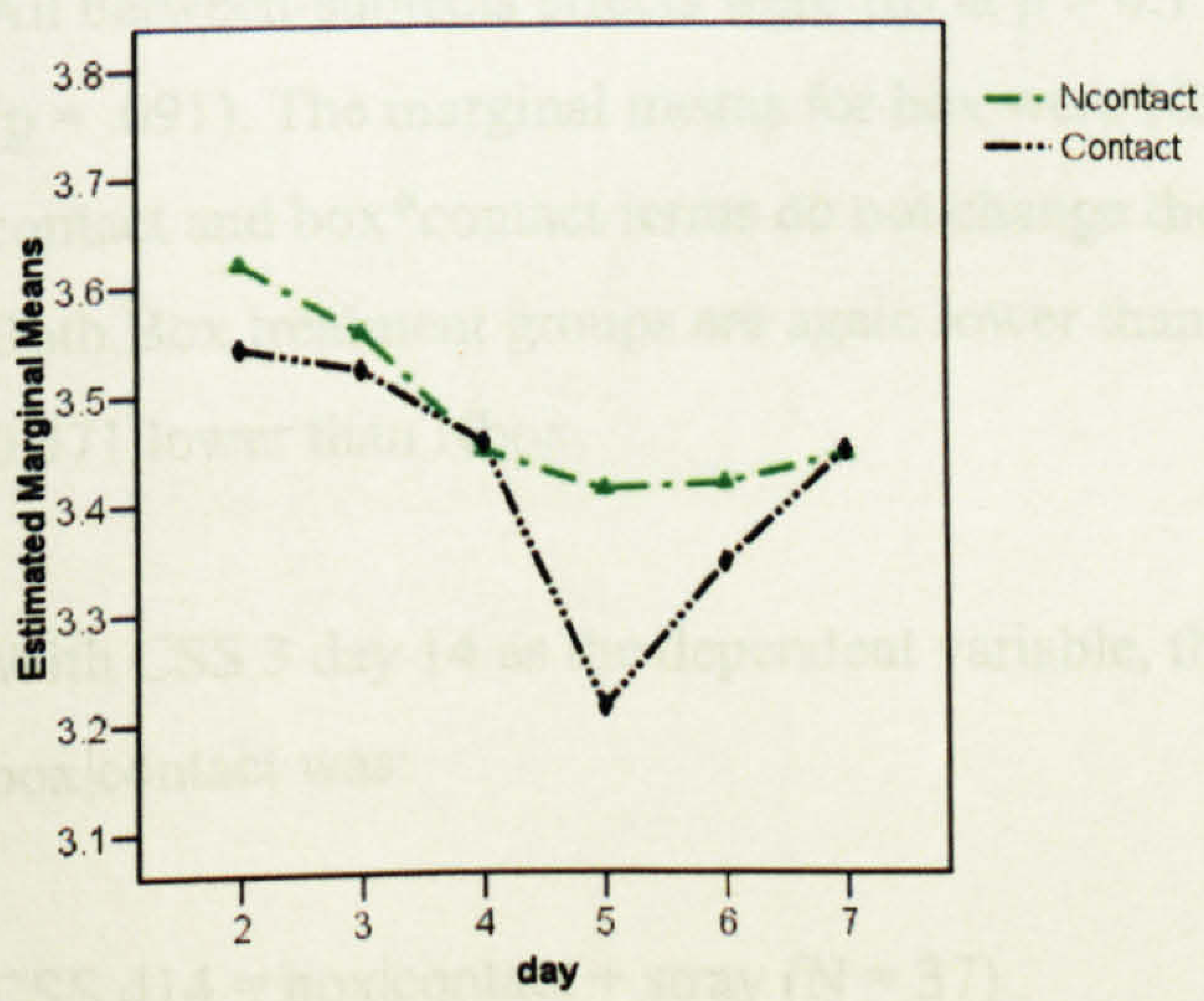
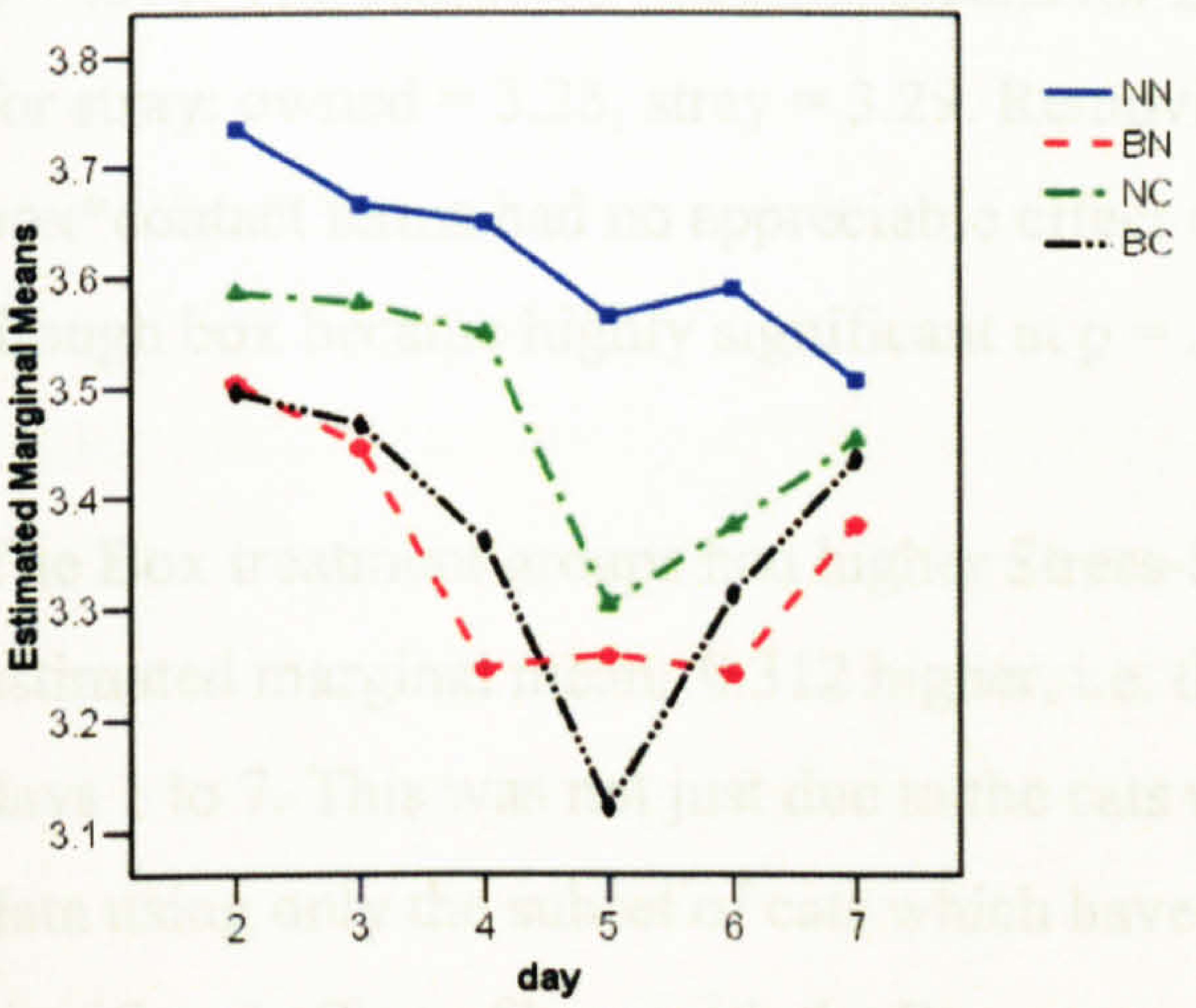


Fig 3.24 Estimated marginal means for CSS, split by treatment group.



For days 2-7, cats given the Box treatment have slightly lower CSS than Nbox cats. This effect is more or less equal across days, with Box cats having an estimated marginal mean of 3.36, 0.19 lower than that of Nbox. This is due largely to the NN group having a particularly high CSS. CSS drops over time, with entire cats having a dip in day 4, though the reason for this dip has not been discovered.

Similar analyses were conducted on the Stress-Scores from the late afternoon measurement on days 1 and 14. With CSS 3 day 1 as the dependent variable, the best equation containing box|contact was:

CSSd1 = box|con + sex (N = 63)

All between-subjects effects were NS at $p > 0.1$ apart from Box ($p = .005$) and sex ($p = .091$). The marginal means for box were Nbox = 3.86, Box = 3.49. Removing the contact and box*contact terms do not change the marginal means for box appreciably. Both Box treatment groups are again lower than both Nbox, with a marginal mean 0.371 lower than Nbox.

With CSS 3 day 14 as the dependent variable, the best equation containing box|contact was:

CSS d14 = box|contact + stray (N = 37)

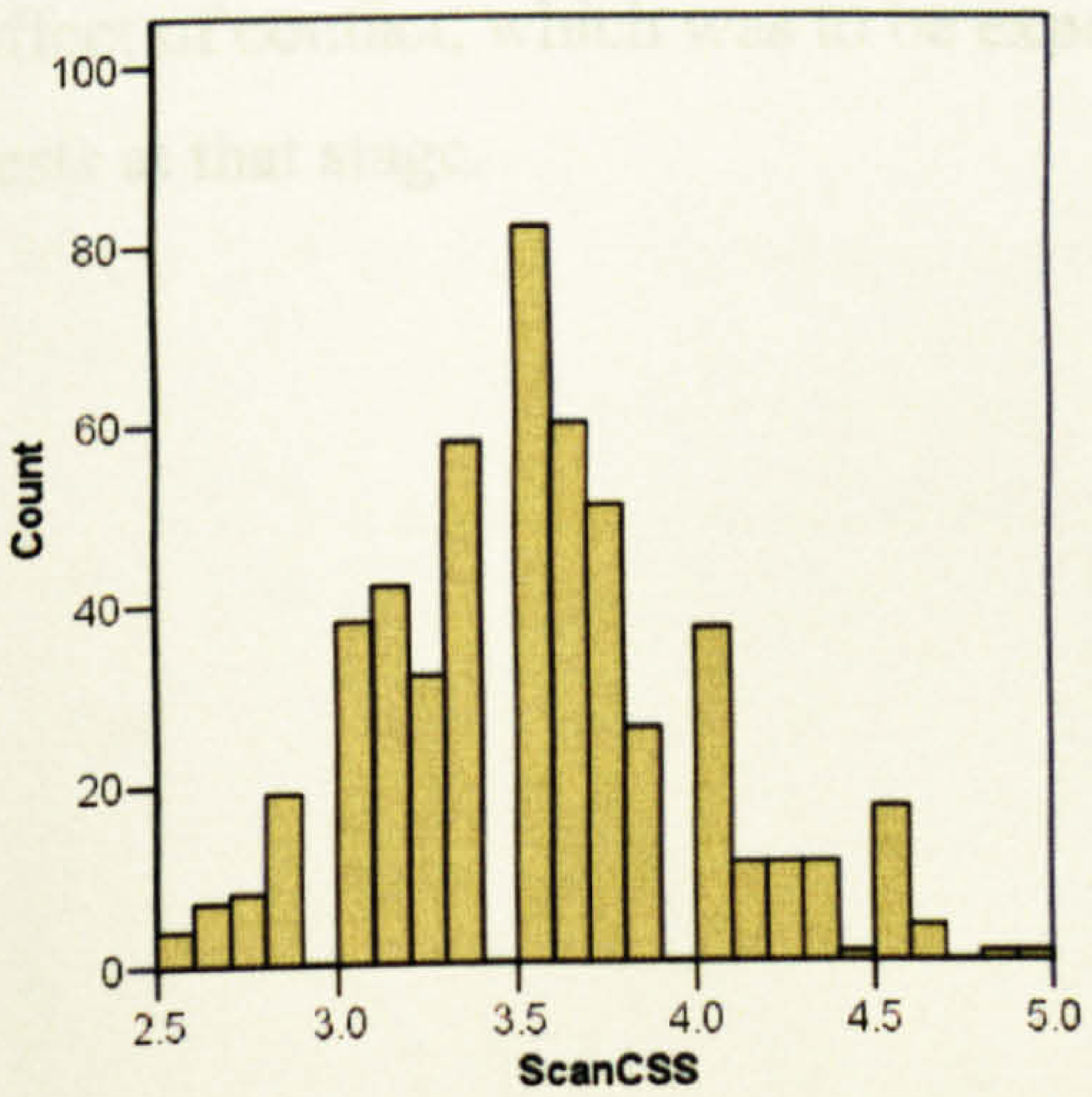
All between - subjects effects were NS at $p > 0.2$ except box $p = .108$ and stray $p = .011$. The estimated marginal means for Box were: Nbox = 3.10, Box = 3.42; and for stray: owned = 3.23, stray = 3.29. Removing the non-significant contact and box*contact terms had no appreciable effect on the estimated marginal means for box, though box became highly significant at $p = .003$.

The Box treatment groups had higher Stress-Scores than the Nbox groups, with estimated marginal means 0.312 higher, i.e. the opposite effect to that observed on days 1 to 7. This was not just due to the cats which had left the data set – testing day 1 data using only the subset of cats which have data on day14, day 1 still had a significant effect of box, with the Box group lower than Nbox (estimated marginal means: Box 0.489 lower). Using the same subset, the Box group is still lower in the repeated measures GLM (estimated marginal means: Box 0.104 lower), though this is now non-significant.

3.3.6 Scan CSS

The most common score for ScanCSS was 3.5 (Fig. 3.25), similar to videotaped CSS.

Figure 3.25 Histogram of mean ScanCSS for all cats, data from days 1-7 (N=521)



The best fitting repeated measures GLM for the dependant variable *ScanCSS* days 2,3,4,5,6,7 was:

ScanCSS d2,3,4,5,6,7 = box|contact

Multivariate tests were significant for day ($p = .000$) and box ($p = .007$), the rest were NS at $p > 0.1$. Within subjects effects were NS at $p > 0.1$ apart from day ($p = .000$) and box ($p = .003$), with CSS falling over day, and falling earlier for Box groups (Fig. 3.26), though there was no significant between-subjects effect of Box ($p > 0.1$). There was a between-subjects effect of Contact ($p = .015$), with the contact group being consistently lower (Fig. 3.27). The estimated mean Contact CSS was 3.392, 0.18 lower than the Ncontact group. Between-subjects effects were NS at $p > 0.1$ for box*soc (Figure 3.28).

With *ScanCSS* 5 day 1 as the dependent variable, the best equation containing box|contact was:

'ScanCSS5 d1' = box|contact (N = 66)

The only significant term was box ($p = .050$). Cats with a box had *ScanCSS* 0.26 lower (estimated marginal mean Nbox = 3.99, Box = 3.73). There was very little effect of contact, which was to be expected as only half of the cats had had any social tests at that stage.

Figure 3.26 Graph of estimated marginal means for *ScanCSS* over days 2-7, split by Box.

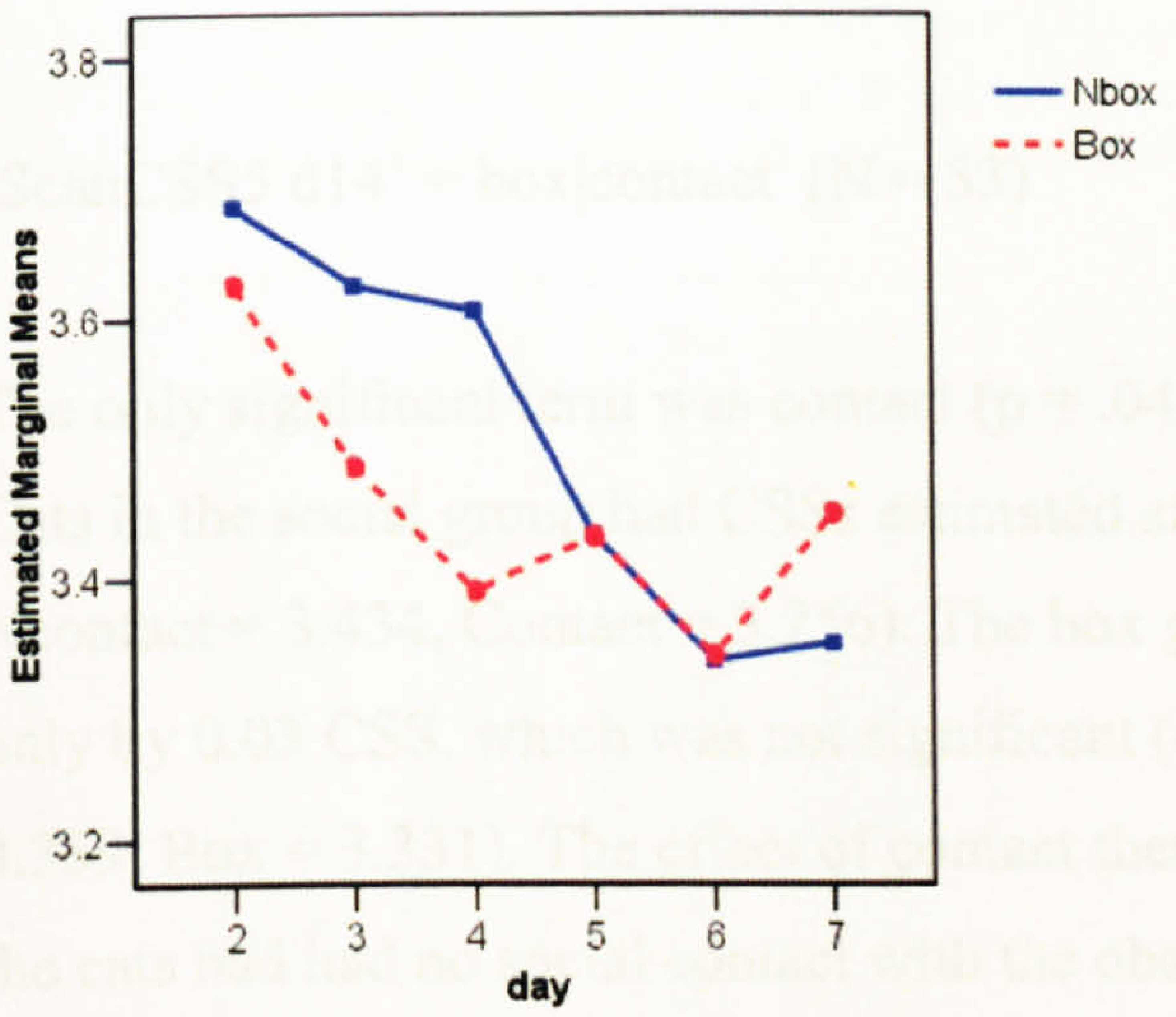


Figure 3.27 Graph of estimated marginal means for *ScanCSS* over days 2-7, split by Contact.

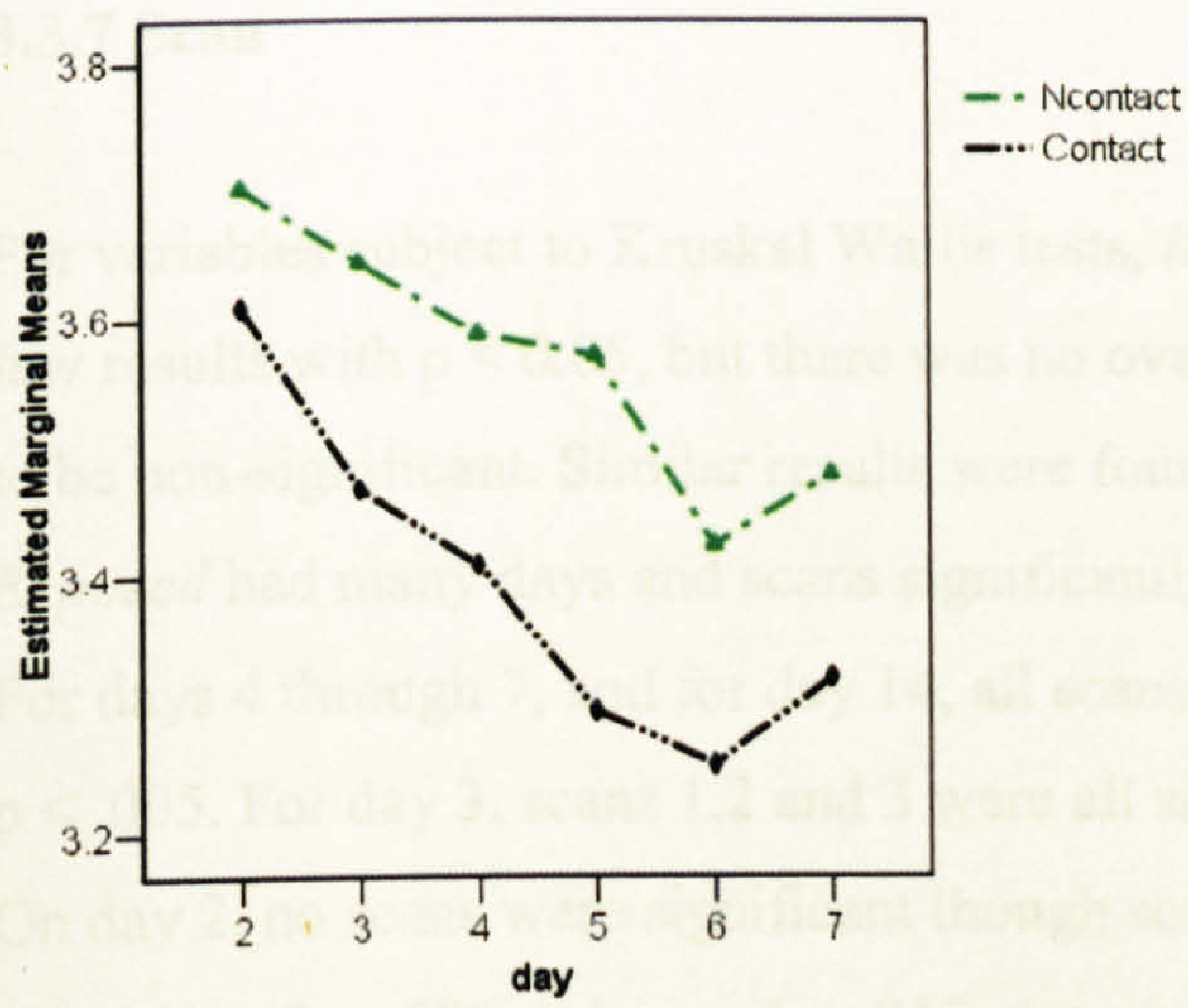
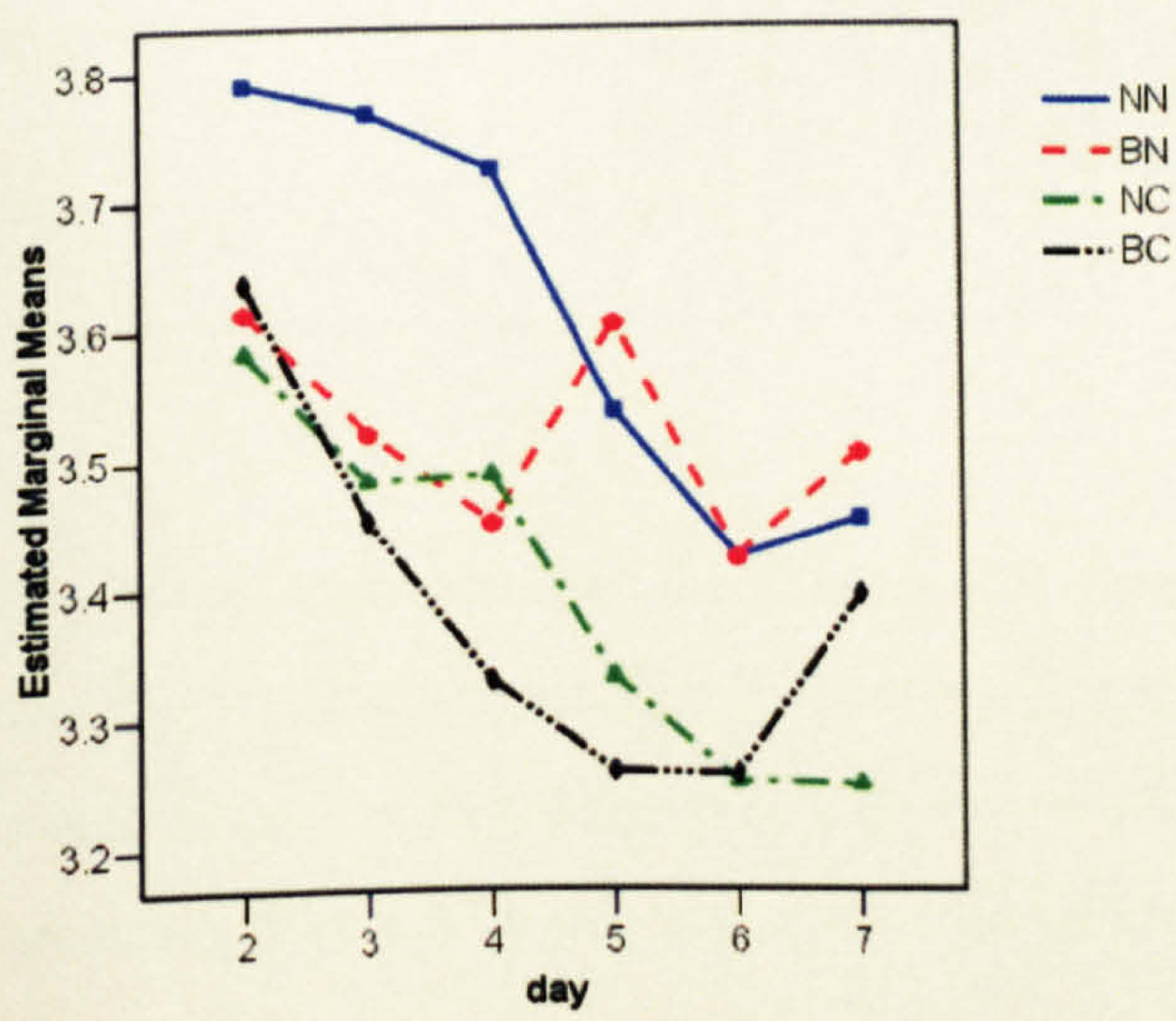


Figure 3.28 Graph of estimated marginal means for *ScanCSS* over days 2-7, split by treatment group.



With *ScanCSS* 5 day 14 as the dependent variable, the best equation was:

'ScanCSS5 d14' = box|contact (N = 33)

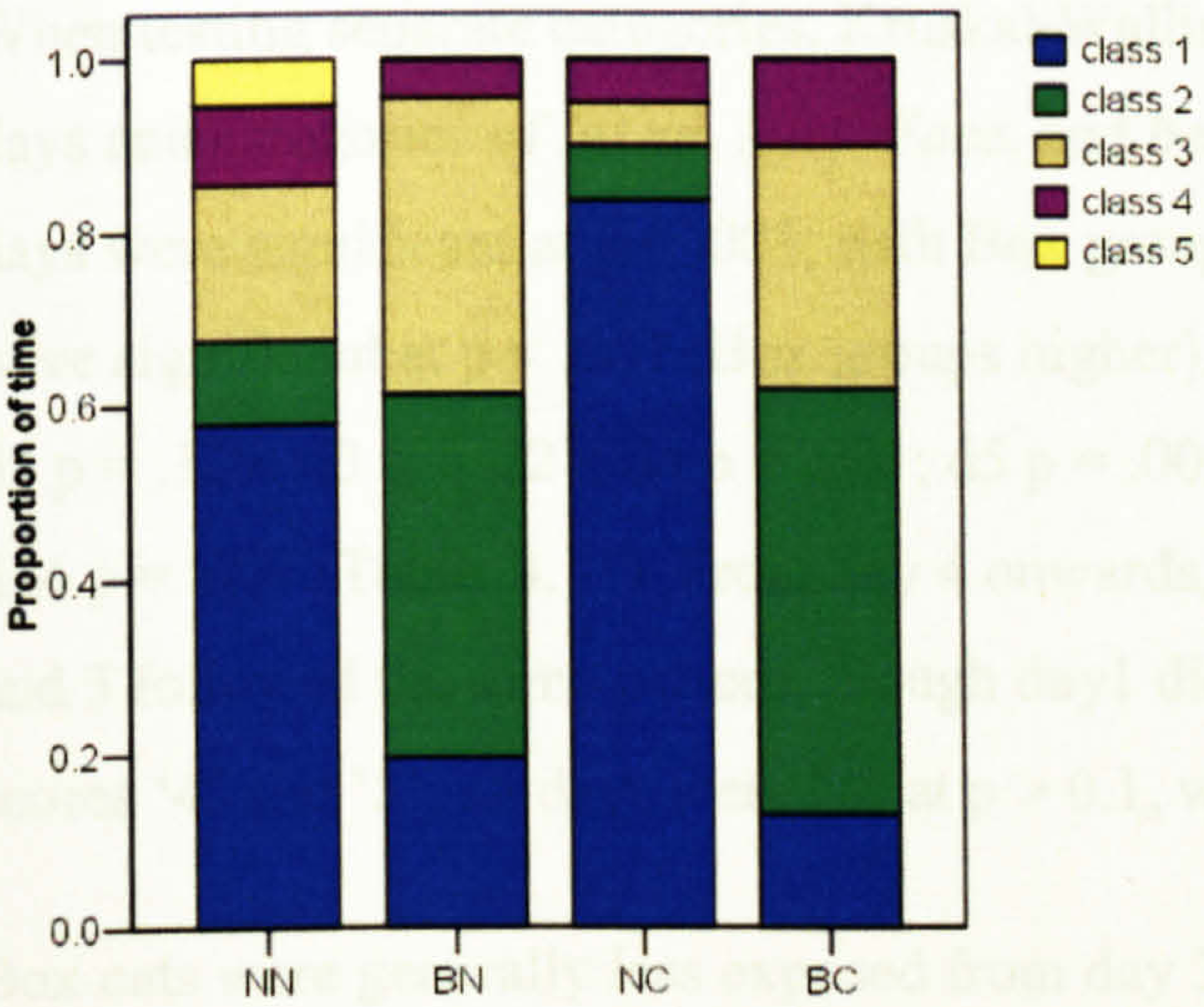
The only significant term was contact ($p = .045$), the rest were NS at $p > 0.1$. Cats in the social group had CSSs estimated at 0.18 lower (estimated marginal means: Ncontact = 3.434, Contact = 3.256). The box group had slightly lower CSS, though only by 0.03 CSS, which was not significant (estimated marginal means: Nbox = 3.359, Box = 3.331). The effect of contact therefore remained to day 14, even though the cats had had no social contact with the observer for at least five days beforehand.

3.3.7 Scan

For variables subject to Kruskal Wallis tests, *inout*, *hfbf*, *face* and *behaviour* all had a few results with $p < 0.05$, but there was no overall pattern to them, so were all judged to be non-significant. Similar results were found with the Chi-square tests of *Position*. *Exposed* had many days and scans significantly different between treatment groups. For days 4 through 7, and for day 14, all scans had $p < .05$, and nearly all were $p < .005$. For day 3, scans 1,2 and 3 were all significant at $p < .05$, scan 4 was .070. On day 2, no scans were significant though scan 1 had $p = .066$ and scan 4 .065. Day 1 had scan 3 = .006 and scan 4 = .047, though day 1 scans 1 and 2 had very small N.

So, from day 3 onwards, both BN and BC had higher *exposed* than the non box groups (Fig 3.29), however this is less true on day 2, as NN is quite high.

Fig 3.29 Timebudget bars for *exposed* on day 4 (a typical day), split by treatment group. Data taken from all 4 scans. Class 1 = In open part of cage, fully exposed; Class 2 = In box / corridor / under ramp (b/c/r), head exposed; Class 3 = In b/c/r, head inside b/c/r but looking out; Class 4 = In b/c/r, head and body inside, looking out; Class 5 = In b/c/r, head and body in and attempting to hide.



For *hide*: day 1 p = .098; day 2 p = .222; day 3 p = .284; day 4 p = .067; day 5 = .006; day 6 = .050; day 7 = .057; day 14 = .067. In all cases (including the non significant ones), cats with a box were hiding more, and the Contact group generally hid less. Taking multiplicity into account, firm conclusions can only be drawn about day 5 (Table 3.10), although it seems likely that the effect occurred from day 4 onwards. Mann-Whitney tests on day 5 comparing Nbox and Box, and comparing Ncontact and Contact were both significant at p = .034 and p = .009 respectively. This confirms the significance is largely due to a low NC result, suggesting a chance occurrence.

Table 3.10 Day 5 *hide* ranks by treatment group, from Kruskal-Wallis test

		Mean Rank
Hide	NN	41.7
	BN	46.4
	NC	25.9
	BC	39.6

So, neither treatment had an effect on whether cats spent time inside the pod or outside, whether their heads or bodies were closer to the front of the pod/run, or on whether they were hiding or not, although it seems likely that Nbox cats hid less (possibly because hiding was more likely to take place when there was something to hide behind), as did Contact cats, possibly due to being better acquainted with the observer. There was no effect of treatment upon their facing, or their behaviour.

When testing separate categories, Kruskal Wallis tests on each day were NS for all days and categories of *InOut*, *Hfbf*, *Face*, and *behaviour*. For *exposed* category '1', all days were significant at $p < .003$, with Box groups lower. For category '2', all days were significant at $p < .007$ (Box groups higher). For category '3': day 1 $p = .776$; d2 $p = .129$; d3 $p = .221$; d4 $p = .001$; d5 $p = .005$; d6 $p = .018$; d7 $p = .000$; d14 $p = .007$ (Table. 3.11). From day 4 onwards, Box groups had higher ranks. Days 2 and 3 followed the same pattern, though day1 did not – NN was highest ranked. For scores '4' and '5', all days were NS at $p > 0.1$, with no obvious tendencies.

Box cats were generally less exposed from day 3 onwards. Looking at individual scores more closely, box group cats spent less time being exposed (category 1) and more time with their body hidden but their head exposed (category 2) on all days studied. Box cats were also more likely to be hidden with their head not exposed but looking out, score 3 (e.g. with their head inside the box, but visible and looking out), though this was only significant on day 4 and later. Days 2 and 3 followed the same pattern, though day 1 did not. So Box cats spent more time being semi-hidden but with their heads visible from admission onwards, and less time in the open. There was no difference between treatment groups in scores 4 or 5 (not looking out of the box/ramp/corridor or actively pressing itself to one side).

Table 3.11 Table of ranks for *Exposed* scores '1','2', and '3', day 4 (typical of all days from day 4 onwards) from Kruskal-Wallis test, split by treatment group.

	Treatment group	N	Mean Rank
EXP_1	NN	19	43.58
	BN	17	27.76
	NC	20	55.80
	BC	19	22.84
	Total	75	
EXP_2	NN	19	27.61
	BN	17	50.97
	NC	20	23.75
	BC	19	51.79
	Total	75	
EXP_3	NN	19	34.95
	BN	17	49.09
	NC	20	25.30
	BC	19	44.50
	Total	75	

So, although Box cats were generally less exposed, they still spent most of their time (60%) either fully exposed or with their heads out of the box / corridor / ramp. There is no increase in categories 4 or 5, so boxes do not make the cats more likely to be non-visible to the public.

Position categories 'in1', 'in2', 'out1', 'out2', 'out3' and 'out 4' were all NS between treatments. *Position* 'in3', 'in4' and 'out5' p-values are in Table 3.12.

Table 3.12 Table of Kruskal-Wallis p-values for *position* 'in3', 'in4', and 'out5'.

	Position 'in3'	Position 'in4'	Position 'out5'
Day 1	.001	.084	1.00
Day 2	.020	.055	.171
Day 3	.231	.114	.117
Day 4	.039	.015	.008
Day 5	.108	.381	.054
Day 6	.482	.227	.045
Day 7	.616	.149	.039
Day 14	.000	.467	.467

For the *position* 'in3' days which have p < .05, box groups had higher ranks, and all of the other days bar one (day 7) followed the same pattern. For *position* 'in4', box groups were lower on day 4, and all other days followed the same pattern, apart from d14 where BN was higher than all the rest. For p*osition* 15, box groups were higher for all days except day 14.

Although the scan tests have problems with multiplicity, each variable or category either has all days non-significant, or nearly all with very low p-values, so interpretation is quite straightforward.

Testing Box and Nbox groups with Mann-Whitney tests had no effect on the NS categories above, but lowered the p-values of categories which were significant above (Table 3.13).

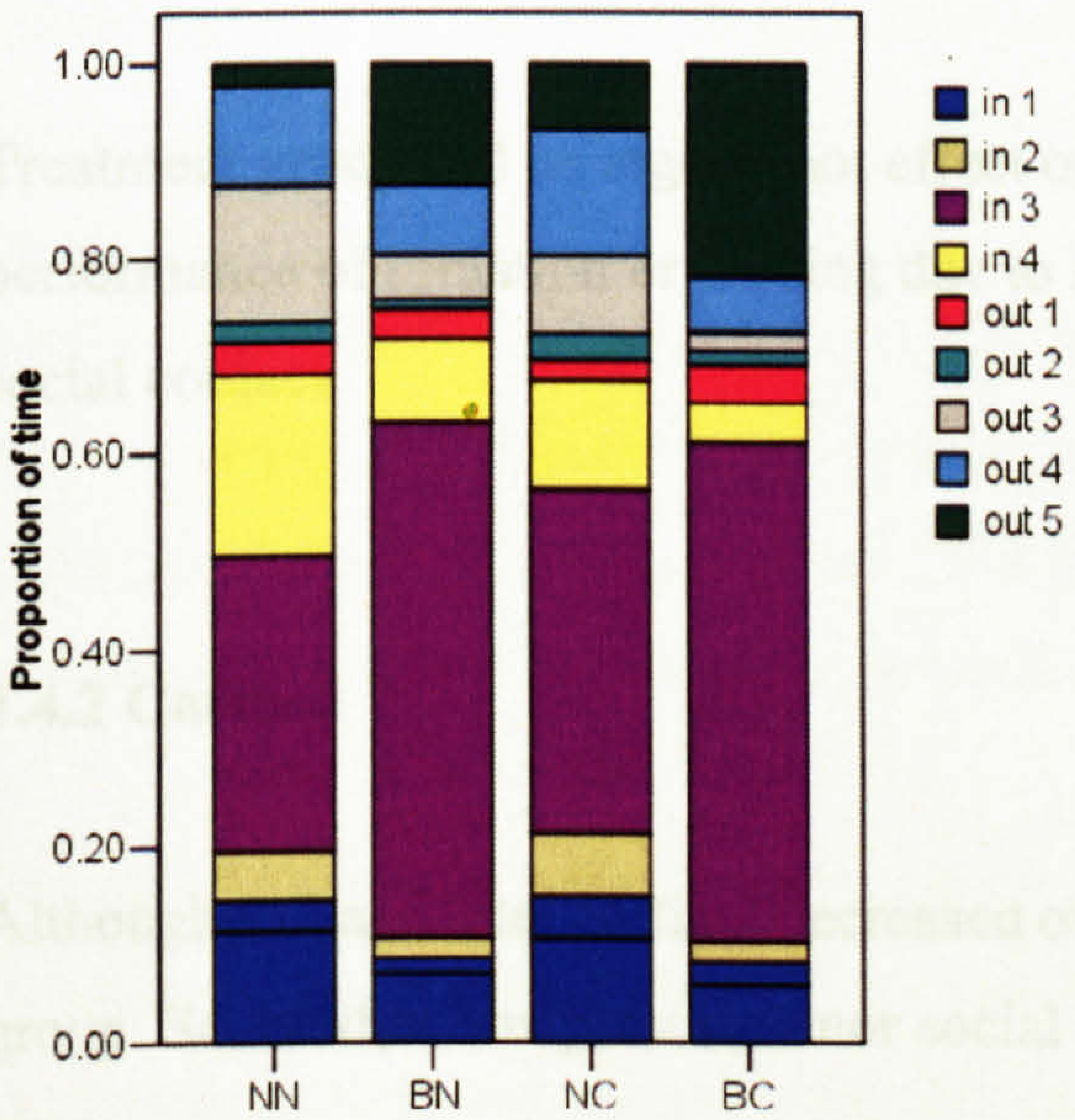
Table 3.13 Table of p-values for Mann-Whitney U-tests, Box vs Nbox groups. * = $p < .05$, ** = $p < .01$. *Position* categories not shown were NS at $p > 0.1$ overall.

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 14
Position 'in3'	.001**	.007**	.054	.004**	.018*	.898	.931	.000**
Position 'in4'	.069	.071	.097	.006**	.148	.042*	.347	.280
Position 'out5'	1.000	.041*	.037*	.001**	.019*	.019*	.007**	.813

Note that day 1 often contained only one or two scans for each cat, so the test will be less powerful. For *position* 'in3', the Box group was higher for all days except days 6 and 7 when Nbox was (non significantly) higher. For category 'in4', Box groups were lower all days. For category 'out15', Box was higher every day except day 1 and day 14, which both had low N (N = 39).

Looking at a time budget graph for *position* (Fig. 3.30), it seems unusual that *position* 'out1', 'out2', 'out3' and 'out4' were not significant in the above tests, as there appear to be differences between Box and Nbox groups. This may be due to the low performance (and hence a large number of zero values) for these the categories, which will reduce the power of the Mann-Whitney and Kruskal-Wallis tests.

Fig 3.30 Time budget graph of *position* (data from all days), split by treatment group. In1 = In pod, front of cage; In2 = back of pod, not in box / boxbed; In3 = Back of pod, in box / boxbed; In 4 = In corridor; Out1 = Out in run, front of run; Out2 = Back of run; Out 3 = Under ramp; Out 4 = On shelf; Out 5 = Back of run, in box / boxbed.



So, there was no significant difference between treatment groups in time spent in the open areas of the pod or run, nor any difference to time spent on the shelf. From Figure 3.30, it looks as though there were differences in these areas, with box cats spending less time in those areas, though this were non-significant.

Box cats spent significantly more time at the back of the cage where the box was, both inside and outside ('in3' and 'out5').The significant differences in 'in3' were in the first 5 days after admission, those in position 'out5' all days in the first week apart from day 1 (which has fewer scans to average from), so the effects of Box on these categories seem quite clear. For 'in4', only days 4 and 6 were significant. Three other days in the first week were close to significance, which strongly suggests an effect of box, with Box cats spending less time in the corridor. The increase in 'in3' and 'out5' which corresponds to box use, would tend to make cats farther away, and so less visible. The reduction in 'in4' suggests that stressed cats use the box in preference to the corridor.

3.4 Discussion

3.4.1 Maintenance

Treatment group had no significant effect on any of the coded variables tested. Non-performance of urination or feeding due to stress was not affected by box provision or social contact.

3.4.2 Cortisol

Although CC and total cortisol decreased over time, there was no effect of treatment group. So, neither box provision nor social contact reduce this physiological sign of stress.

3.4.3 Approach test (unfamiliar female person)

Approach generally decreased over time as cats became accustomed to the shelter. It is interesting that most cats become no more likely to approach between days 7 and 14. This may be due to the cats having plateaued in their *approach* by day 7, or it may be that having unknown humans observing cats in rehoming all day might inhibit any further decrease in *approach*, or that constant exposure to the observer is necessary to cause a decrease.

Neither of the treatments had an effect on approach, though there was an effect on the change in *approach*, with the Box group less likely to have an increase / more likely to have a decrease. This was not due to the box group having greater *approach* on day 3 and therefore having more ability to decrease. Although it is not clear from the data what is driving this decrease, it seems likely that the Nbox cats took longer than the Box cats to decrease to plateau. This suggests a positive effect on rehomability of box provision, though this cannot be confirmed. It is possible that the reverse is true, with

Box delaying a decrease, though the (non-significant trends) in the data do not support this.

The magnitude of the effect is hard to quantify as *approach* is ordinal and the median for both groups of change in *approach* is zero (no change). From the ordinal regression on *approach* day 3 – *approach* day 7, the parameter estimates for *approach* day3 (-3.31, -2.27, -3.02) were far higher than the estimate for Nbox (.690), so a cat's previous approach test was far more informative about future approach tests than knowing whether it had a box or not (though this previous test would include the effects of box as well as the cat's personality).

Strays were less likely to approach, which may be due to some being poorly socialised to humans (this being a factor in why they became strays), or unaccustomed to human contact since they had become stray. Older cats tended to score higher on all days tested, though this was only significant on day 14. This may be due to young cats being more easily excited, and old ones less likely to get up and move around - since most cats who moved approached the observer, most movements tended towards lower *approach* scores.

3.4.4 Social (familiar male person)

No clear effect of treatment group on any of the variables was found. Approach test and the *soc1st* values decreased between days 3 and 7, so cats became more likely to approach a known human, and more friendly over time. There was no difference between day 7 and day 14 values, so the cats may have plateaued in their reactions to the author by day 7, or had no change due to the time without social contact between days 7 and 14. The high NN*firstapp* on day 3 may have been due to an effect that only occurs when cats are acutely stressed shortly after admission to the cattery, but equally well may have been due to chance.

This lack of effect shows that although cats with boxes were more likely to remain at the back of the cage, they were no less likely to approach a known observer. In comparison to the staff approach test, the lack of an effect of Box group on *firapp* or *soc1st* may indicate that previous experience of the observer may be far more important than any general effect of stress. So, boxes may not make cats less likely to approach, or less friendly to, a known observer. Together with the staff approach test, this confirms that boxes do not make cats any less likely to approach a human, nor any less friendly. There was no effect of Contact – possibly all cats, including Ncontact cats had had enough contact with the observer by day 3 to treat him as a known human in the approach test. The lack of difference in *friendliness* shows that Ncontact cats are no more or less 'keen' to obtain social contact at the start of the test.

Strays and older cats were less likely to approach, the same as the staff approach test. This was not significant on day 14, though the lower N reduces the power of the test – the parameter estimates were the same sign as on days 3 and 7.

3.4.5 CSS

Boxes reduced cats' CSS scores by an estimated mean of 0.19 over days 2-7. This effect was most pronounced just after the cat enters the shelter (day 1 Box CSS is estimated to be .371 lower than Nbox CSS at CSS3 day 1). From Figure 3.24, this additive effect remained for the first 5 days, then may have started to decrease by day 7. A decrease of nearly half a CSS score on day 1 may be quite a welfare improvement, and for most cats will reduce them from 3.86 (very tense) to 3.49, which is between 'weakly tense' and 'very tense'. The general reduction of 0.19 on days 2-7 is smaller, though since over 90% of all CSS scores on these days lie between 3.0 and 4.0, this is a reasonable proportion of the variation. How much this difference relates to the cats' internal state is unknown, though a reduction in CSS away from 'very tense' towards 'weakly tense' should indicate an improvement in their welfare.

This effect did not continue to day 14 however, where Box cats had CSS 0.312 higher than Nbox. This may be because boxes were generally rare amongst cats in homing, and may have attracted attention from the public. Anecdotally, the public were observed to sometimes lean closer in an attempt to see Box cats better, which may have caused them to become more stressed. Since the boxes allow only semi-hiding, the cats cannot hide completely when the public do this, and this extra / closer attention might make them feel less secure then Nbox cats. The effect of boxes on humans who come in to observe the cats has to the author's knowledge not been investigated to date. It is instead possible that cats with boxes in homing became used to people passing them by because they were not seen, so the sudden close scrutiny by a video camera might alarm them more. This appears to be in direct opposition to the previous hypothesis, though it is possible that box provision makes a cat looked at less often, but more closely scrutinised when it is.

Although it is possible that the effect of Box might be unique to observing cats with a video camera, this seems unlikely. It may have affected early CSSs as any novel stimulus would, possibly being an acutely aversive stimulus which cats felt safer being able to hide from. Few cats appeared distressed by it, and seemed to acclimatise to it after a few scans – since the camera did not predict an entry into the pod by the observer (if anything, quite the opposite) there was no reason for cats to remain fearful of it, and the effect of box continued to days 6 and 7.

Older cats had higher CSS than younger cats, though this was quite a weak effect, and only present in the repeated measures analysis – day 1 and d14 had NS effects of age, and the sign of estimated effect was negative, with older cats having lower CSS. The effect of age is still unclear. Stray cats had lower CSS than owned cats, though this was only significant on d14 cats, possibly due to the increased public presence around them. The same trends (though NS) were present in repeated measures GLM results, though were not significant with a low effect of .061, only just outside the standard error. Although there may be a general trend, it is largely unimportant even if true.

Given the positive correlation between CC and CSS in Chapter 2 (within individuals, Table 2.13), the lack of any treatment effect on CC is odd. It may be due to there being less effect of Box or Contact from 6pm-8am – since cortisol will be secreted over this time, this may reduce the effect (from Fig 3.7, both Contact groups have lower CC, though this is NS).

3.4.6 ScanCSS

ScanCSS fell over time, with Contact cats having lower *ScanCSS* then Ncontact cats, even into day 14 when the cats would have had no social contact with the observer for at least 5 days. This reduction was by 0.18 for days 2-7 and day 14, similar to the effect of boxes on videoed CSS. From the graph of estimated marginal means (Fig. 3.26), the effect is more or less additive, though a little reduced on day 2, when the cats had not yet had much social contact. Social contact made the cats seem less stressed when observed by a known human, though it had no significant effect on the videotaped CSS.

None of the other variables had a significant effect except for Box. There was an effect of having a box on day 1, with box cats having a lower *ScanCSS* (0.26 lower). Although Box had no between-subjects effect on *ScanCSS* after day 1, Box cats decline to plateau a day earlier than Nbox cats (Fig. 3.25). Together with the day 1 decrease in *ScanCSS*, this suggests that Box has an effect when the cats are still acutely stressed by entry to the shelter, but that the effect is not present once cats become more acclimatised to the shelter around day 5.

Since social contact made the cats seem less stressed when observed by a known human, it had no significant effect on the videoed CSS. This suggests that boxes may have a more global effect on stress, reflected in videoed CSS and the earlier reduction in *ScanCSS*, but that this effect is largely replaced in *ScanCSS* by the cat's reaction to the observer, showing how important humans are as stimuli. Also, since the observer entered each cage and attempted to interact with the cat regardless of whether the box was present or not, the cats will have learned that the box offered no protection against him. This may have reduced the effect of box when the observer was present. It is possible that the box does not affect responses to stress caused by humans, and so may not help stressed cats as much once transferred to homing (which may help explain the day 14 result in CSS, above).

Cats' views of their human caretakers as either pleasant or unpleasant stimuli may be important for their welfare. This is also the first positive evidence for an effect of the social contact – even though increased contact did not seem to affect cats' *approach*es towards the observer or unknown humans, how friendly the cats were during social tests, or videtaped CSS, increased social contact does seem to reduce cat's stress when that known human is present. Whether this effect would be extrapolated to other humans is not known as all CSSs were taken by the observer, though it seems likely that it is an effect unique to the human who provided the social contact, with social contact making him seem less threatening, or seen as a pleasant stimulus which temporarily reduces that cat's stress.

3.4.7 Scan

Boxes and social contact had no significant effect on whether a cat faced towards the public or not, or on how active and alert the cat was. This assuages any concerns that boxes will cause cats to be less alert, or face away from the public. However, boxes may make cats hide more, though only on day 4 or later (once cats have become somewhat accustomed to shelter life). This result was not quite significant, and is something that deserves more research.

Box cats were generally less *exposed* from day 3 onwards. They spent less time fully exposed (category '1') but more time in the box with their head exposed (category '2') on all days. Box cats also spent more time with their head slightly more hidden but still in view (category '3'), though this only differs from the Nbox groups from day 4 onwards - stressed Nbox cats who are category '3' in the initial few days may have less motivation to be hidden from day 4 onwards, so decrease in this level of exposedness. Box cats might not show this decrease either because the box makes it very easy for them to have a score of '3', especially if the cat is small, or because the box keeps them more fearful (possibly by reducing exposure to, and thus habituation to, unpleasant stimuli). The first conclusion is better supported by the data, since Box cats have a lower CSS than Nbox. There was no difference between treatment groups in categories '4' or '5' (not looking out of the box/ramp/corridor or actively pressing itself to one side). These are the two *exposed* scores that indicate most motivation to hide from the surroundings, and may affect homability, so the lack of significant increase in Box groups is a good sign that boxes may not affect homability to a great extent.

Boxes have no effect on most *position* categories, as there was no difference in time spent out in the open parts of the pod or run, or on the shelf. Box cats did spend more time in the box area of the cage than Nbox cats, both in the pod and out in the run. So, cats used boxes when available. The effect of boxes on exposedness was dealt with above, though this result indicates that cats utilize the boxes, indoor cats spending around 125% of the time Nbox cats do in that area of the cage, and around 300% for outdoor cats (Fig 3.30). This large increase for outdoor cats was accompanied by a (non-significant) decrease in hiding under the ramp. Since it was cold outdoors for much of the study, cats may have only hidden outdoors when the box provided a little shelter. The possible decrease in time spent in the corridor, indicates that boxes are preferred hiding places. Since corridor use makes cats appear cramped and obviously stressed, (the corridor is quite small compared to an average cat) this might indicate a benefit to homing, though this was not significant in day 14 cats.

There is no effect of Contact on any scan variable, again suggesting that it has a quite specific effect rather than a more global one.

3.4.8 Overall

Boxes are used by cats when present, and appear to reduce the acute stress caused by transfer to a shelter. The effect is greatest on day 1 and reduces over time, which suggests that shelters wishing to use boxes should put them in a pod as soon as the cat is admitted (preferably before admission, so they do not form a preference for another hiding place before the box is introduced). Boxes affect stress quite generally, while social contact with a known human may only affect stress when measured by the human who gave the contact – i.e. contact only has an effect when that individual human is present. This effect is confounded with gender – while the author was male, all staff volunteers for the approach test were female. This is unlikely to have had an effect at the population level, and all cats were exposed to both male and female caretaking staff. Anecdotally, some cats at shelters are reported to be fearful of men. This is rare, and no instances have been reported to the author of fear towards women (though since many catteries are staffed solely by women, such an effect might be misinterpreted as general fearfulness).

While this effect of Contact might suggest that it is preferable for staff to spend a little extra time giving cats boxes, cleaning them etc, than for staff to spend extra time with the cats, stress caused by caretaking duties was not examined, and may be important to the cats. The benefit of the box seems to only apply in the first week, and may not help cats in homing at all, as they may attract attention of the public. This may not be a problem if boxes that allow better hiding are used, so that cats feel more secure in the box even if it encourages the public to look at the cats more closely.

While it is beneficial for staff to spend time with the cats, the results suggest that social contact with humans who are not part of the regular caretaking staff may not help to reduce the cats' acute stress. Shelters frequently have 'cat cuddlers', human volunteers who come in and give the cats social contact. This study suggests that their spending time with cats shortly after admission may not be particularly useful for reducing their stress, though the effect on chronic stress is unknown. Most cats appear to enjoy this contact, so there is a welfare benefit to it, though this may be acute rather than long term.

These differences between treatment groups suggest that CSS recorded remotely is a more global measure of stress, while ScanCSS measures not only that but how the cat is reacting to a human outside its cage, with the reaction to the human being more important than the 'global' measure. This suggests that any future studies employing human-recorded CSS should be careful to minimise observer interaction with cats, and make what interaction there is the same for all cats studied. It also raises the point that any human-scored CSS will probably tend to decrease over time, as the cat will become more relaxed in the observer's presence.

There was no effect of social interaction on the staff approach test, which suggests that cats do not necessarily 'extrapolate' good experiences with one human to other humans. However, there was also no effect on the observer approach test, or the friendliness test in the first minute. This does not necessarily show no effect of the social contact – whether cats approach or not, and how friendly they are, may be as much a part of that cat's personality as their experience with that human, and the one minute's social contact with Ncon cats may have been enough to get them accustomed to the author's presence and become more likely to approach over time. Even Ncon cats would have seen him walking up and down the corridor, interacting with cats opposite, etc, so this and the one minute's social interaction may have been enough for them to have no difference in approach tests.

Cats with boxes do spend around 60-70% of their time inside or partially inside the boxes (both inside and outside), more than Nbox cats spent in their boxbeds, and are generally less exposed. Although it is this that seems likely to be reducing their stress score, it may simultaneously affect their homability. Cats with boxes are less likely to hide in the corridor – this is very cramped for most cats (which may be partly why they hide there) but offers little visual occlusion from humans passing by, so hiding there may indicate an acute welfare problem. It should be noted that, although less exposed, the exposedness scores with significant differences (2 and 3) allow the cat's face to be seen clearly, there was no significant in scores 4 and 5, in which the cat's head is hidden, so may affect homing little. So, although boxes have no negative effects in admissions, there may be reason to remove boxes once the cat has been moved to rehoming, preferably after the first few days, when the stress of the move has died down.

Strays were less likely to approach in both the staff approach test and the observer approach test. This may be due to many stray cats being poorly socialised to humans, or being unused to regular handling. There was no indication that strays had higher CSS in either video or scan tests however – the only significant result was d14 CSS, with strays having slightly higher CSS.

Chapter 4: Experiment 3 - effects of providing long-stay cats with boxes

4:1 Introduction

Chapters 2 and 3 concentrated on the acute stress caused by moving into a new housing system. To complement these, Chapter 4 investigated whether boxes can affect the welfare of cats which have been at a shelter for a long time. At this point, the stressors of being in a strange place with a very different lifestyle to that the cat was used to are presumably largely gone – the cat knows and can predict the daily routine of the shelter, and knows the shelter staff and most of the neighbouring cats by sight and smell.

Many stressors still remain, however, and each cat may perceive them differently. These may include the presence of other cats, a lack of attention compared with their previous home, the confined space, the presence of unfamiliar humans during the times when the shelter is open and, especially for poorly socialised cats, the presence of known humans. Some cats remain very tense (CSS 4 or above) after a month at the same shelter, so these stressors may still be a welfare problem for some cats, and may reduce homeability.

Ways to reduce stress in the long-term are therefore still of interest to shelters. Smith *et al.* (1994) conducted a series of enrichment studies on long-stay cats and found that they did use platforms and hiding places when provided. However, the study did not check whether this use actually helped the cats to cope with stressors in their environment.

Some shelters are unwilling to give cats boxes in the belief that a cat hiding in a box is less likely to be adopted than a cat that does not have the option of hiding away when the public want to see it. This problem is of particular concern to shelter managers once cats have reached the rehoming area of the shelter.

This study provided long-stay cats with an enrichment, in the form of a box they can hide in and perch on that also allows the public to view the cats. The aim was to see whether the provision of this box caused a decrease in stress, and whether it affected visibility to the public.

4.2 Method

4.2.1 Subjects

Cats were studied at three shelters – Bath Cats and Dogs Home (a branch of the RSPCA); the Cats' Protection National Cat Adoption Centre (NCC) near Gatwick; and Axehayes shelter in Exeter (also a branch of Cats' Protection). All cats were single-housed, in good condition and free of serious illnesses during their observation. Cats less than 1 year old or over 15 were not included in the study. All cats had been neutered at least a week before the start of the study, and were a mixture of ages, sexes and owned / strays (Table 4.1 and Figure 4.1). The mean age was 5.6 years for all shelters, 4.5 at Bath, 6.1 at NCC, and 5.6 at Axehayes. All cats observed were in the rehoming area of the shelter, and had been in the same pod for at least a week before the study started.

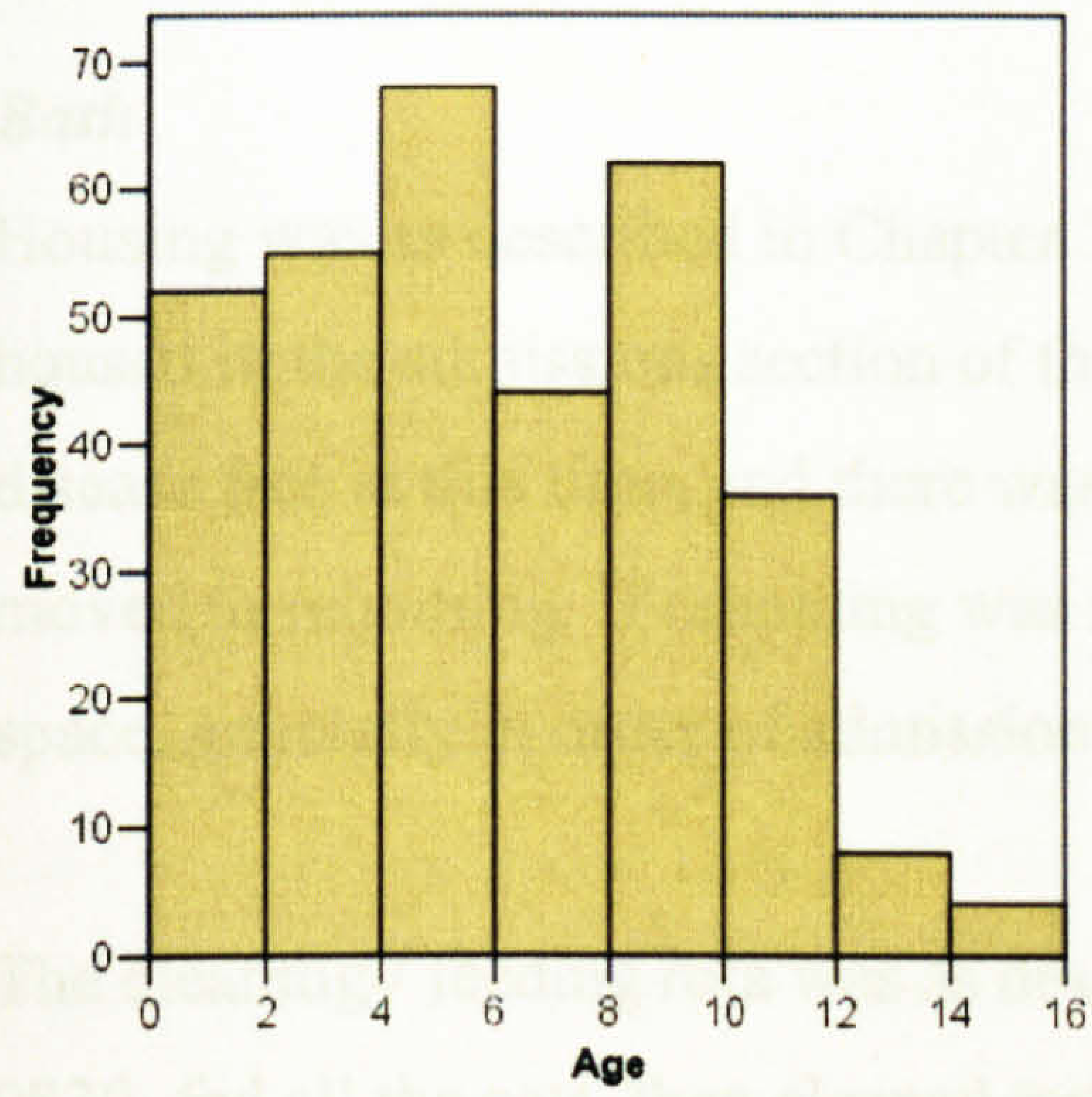
Over a hundred cats were observed initially, though as some became ill or were rehomed, only 89 made it into the final analysis – 11 at Bath, 46 at NCC and 32 at Axehayes. Each study was carried out over one preparation day (Wednesday, day 0) and four experimental days (Thursday - Sunday, days 1 - 4) at each shelter sequentially, between 1st and 19th December 2004: Bath 1-5th, NCC 8-12th and Axehayes 15 -19th.

Female, owned cats formed a large proportion of the dataset, and there were twice as many females as males in this sample. This is more skewed than in previous chapters, where the ratio was only slightly skewed towards females.

Table 4.1 Cats observed at the three shelters.

	N	Female : Male	Owned: Stray	Male, Own : M, Stray : Fem, Own: F, S
All shelters	89	58 : 31 (0.65F)	62 : 21 (0.25S)	17 : 12 : 42 : 9
Bath	11	7 : 4 (0.63F)	5 : 4 (0.44S)	1 : 2 : 4 : 2
NCC	46	33 : 13 (0.72F)	35 : 7 (0.17S)	9 : 3 : 24 : 4
Axehayes	32	18 : 14 (0.56F)	22 : 10 (0.31S)	7 : 7 : 14 : 3

Figure 4.1 The number of cats of each age in the study



There were few cats over 10, otherwise all ages were represented. There were even fewer cats over 15, which may indicate an under-representation of older cats in shelters. However, anecdotally some shelters will often list a cat's age as younger than it probably is, to assist it in being rehomed.

Nearly all cats were domestic shorthairs (DSH), with a few domestic semi-longhairs and domestic longhairs, similar to the previous experiments.

Just over half of the cats with stated preferences liked other cats (26 versus 19 who did not like other cats), as judged either by their owner, or by staff after admission. Axehayes kept data on what background their cats came from, and out of 31 cats with such data, 21 were from urban backgrounds, 9 from semi-urban and only 1 from a rural background.

For NCC and Axehayes, the author took data on the size of cats, judged categorically. Of the 78 NCC and Axehayes cats, 14 were 'small', 28 'medium', 31 'large' and 5 'very large'. There were more 'large' cats than I had expected from previous studies. The boxes still offered enough space for these cats, though for the 5 'very large' cats the box was quite confined and they could not fit into the box without assuming a curled posture. For two of these cats, the entrance hole in the side wall was enlarged so they could fit through.

4.2.2 Housing

Bath

Housing was as described in Chapter 3. Upon being admitted to the shelter, cats were housed in the admissions section of the cattery for at least one week. If they were disease free at this time, and there was space in the rehoming section, they were moved to rehoming. If rehoming was full, cats were moved there once there was space, generally in order of admission.

The cleaning / feeding rota was as described for Chapter 3. Briefly, staff arrived at 0830, fed all the cats, then cleaned out the pods and replaced used litter trays with fresh ones. This took until 1030-1045. The shelter opened to the public at 1100 and closed at 1600, followed by further feeding and replacing of used litter trays.

NCC

Upon admission to the shelter, cats were housed in the admissions block for at least seven days (strays for two weeks). Members of the public were not allowed into this area of the shelter, and rarely walked close to the outside runs.

When there was space, cats were then moved into the prehoming block. Members of the public were allowed into this block, but were escorted by a member of staff. When there was space, cats were then moved into rehoming, where they remained until homed. There were two rehoming blocks, left homing and right homing, both open to the public.

The purpose of shelters having a separate 'prehoming' block is to take cats away from admissions quickly (as there is a greater disease risk there). They are not moved straight into rehoming so that the cats currently in rehoming (which have generally been at the shelter for longer) are seen by the public first. However, the NCC was unusual in that it had opened in 2004, and was partially filled with cats from other Cats Protection shelters. As a result, there was not much difference in time spent at the shelter between cats in prehoming and rehoming. Since nearly all visitors visited both homing and prehoming, and cats were often moved straight from admissions into prehoming, cats from both areas were observed for the study. Similar numbers of cats came from each block (Left homing: 15, Right homing: 14, Prehoming: 17).

The rehoming blocks were identical, and each consisted of 36 indoor pods in two rows of 13 along each wall (Fig. 4.2). These pods measured 0.8 x 0.8 x 0.8m, and the bases were 0.9m off the ground (Fig. 4.3). The inside surfaces and back wall were opaque grey plastic, and the entire front of the cage was clear glass through which the occupant could see cats on the other side of the corridor. The entire floor surface of the pod was covered with Vetbed (a proprietary blanket type material, designed to stay mostly dry if urinated on, and easily washable). Each pod was furnished with a few toys, but was otherwise empty. A cat-flap in the back wall gave the cat free access to the outside runs and was surrounded by a window so the cat could easily see out into the run. The pods had constant underfloor heating.

Figure 4.2 NCC corridor, rehoming.

Figure 4.3 NCC pod, rehoming

The outside runs were fully covered, and although the public were allowed access to them, few people did so. The runs were 1.0x1.9x2.1m (WxDxH), floored with concrete (Fig. 4.4). The end by the corridor (furthest away from the indoor pods) terminated in a metal grille door, through which staff entered for cleaning. The cat-flap from the inside pod led into a corridor (0.36x0.25 x0.25m) which went through the outside wall into the run, to which cats were allowed continual access. The corridor led out on to a broad shelf 0.35m wide which ran the length of the back wall. A ramp led down from the shelf to the floor, and there was one other small shelf high up on the back wall, reachable only by jumping, 0.40m above the main shelf (1.2m above floor height).

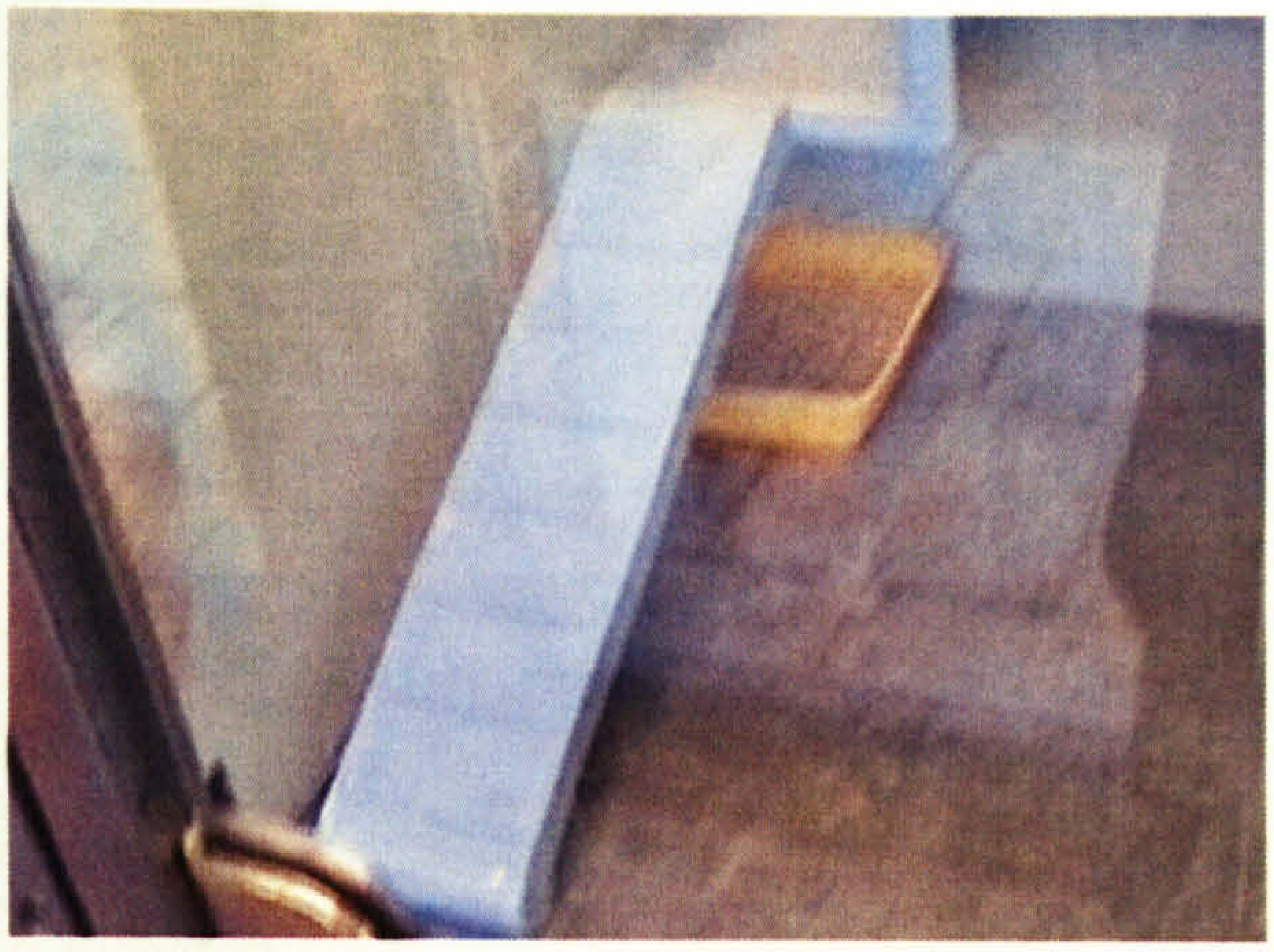



Figure 4.4 NCC outside runs, rehoming. (Left) shelf level, (right) at floor level.

The side walls were opaque frosted glass, though there were two sets of unfrosted glass 'windows' through which cats could see cats in the adjoining pens. One set was at floor level at the end of the ramp, by the door. The other was either side of the main shelf. Food and water bowls were on the main shelf, with the litter tray at floor level underneath. Cats were occasionally observed interacting through the glass, either peacefully (trying to sniff each other, rubbing against the glass), or aggressively (one cat presents an aggressive display to the other and/or tries to attack through the glass).

Prehoming pods (Fig. 4.5) were identical but slightly lower (0.7m off the ground), and the outdoor runs were identical but with the main shelf lower as well. To compensate for the general decrease in height, a second small shelf was fixed on the back wall, 0.7m above the main shelf and 0.3m above the first small shelf.

Figure 4.5 NCC prehoming corridor.

The daily routine was the same for all 3 blocks. Staff started at 0800 with feeding the cats and changing litter trays. While this was happening, part time cleaning staff cleaned inside the pods and around the blocks. The Vetbed was taken out so the floor of the pod could be washed, and timid cats often retreated to the outdoor corridor while this was taking place. The same Vetbed was generally put back in unless dirty. Cleaning usually finished by 1000, when the public was allowed access. The public could only enter the pods with a member of staff present. The shelter closed to the public at 1600, and the cats were fed again, and litter trays changed if dirty. The staff were generally finished by 1645, and the lights turned out. Staff were sometimes swapped from one block to another, to get a feel for how the entire shelter worked (the shelter was opened only recently). Cleaning staff generally stayed in the same blocks, but had little time to spend with the cats.

Axehayes

Upon admission to the shelter, cats were housed in the admissions block for one or two weeks. Members of the public were not allowed into this area, and rarely walked near to the block's outside runs.

When there was space, cats were moved to the prehoming block so long as they were well. Members of the public were not allowed into this block. For this reason, cats in prehoming were not studied at Axehayes.

Cats typically remained in the prehoming area for 3-4 weeks, until there was space in homing. The homing area consisted of two linked corridors: Rescue 1, which consisted of 29 pods along both sides of the corridor, and Rescue 2, which consisted of 18 pods (Fig. 4.6). A further homing area of 10 pods ("Oldies") contained some of the older cats at the shelter. "Oldies" was just outside reception, a door to one side led to Rescue 1, which led on to Rescue 2.

Figure 4.6 Axehayes Rescue 1 corridor. Rescue 2 and 'Oldies' were similar.

All the pods were similar: they were 0.8 x 0.9 x 0.9m (WxDxH), and the bases were 1m off the ground. The inside walls and ceiling were painted concrete blocks, with a floor of plastic (Fig. 4.7). At the front of the cage, a glass door 0.6m across provided access for staff and allowed the cat to see out, and to observe and be observed by cats opposite. The back wall of the pod was entirely glass, with a cat-flap at the back leading to the outside run. Each pod contained food and water bowls, and an oval cat bed. The beds were of varying sizes but were around 0.6m wide, 0.4m deep and had walls 20cm high. At the front of the bed, the wall was only a few centimetres high so the cat could enter and exit easily. Each bed had a heatpad, over which a Vetbed blanket was laid. The heat pad was constantly on at that time of year. The side of the cage which had the cat-flap on differed between the two sides of the corridor, so the pens were nearly mirror images of each other. The floor was uncovered.

Figure 4.7 Axehayes, inside pod

The outdoor runs were fully covered (Fig. 4.8), and although the public were allowed access to them, very few people did so. The runs were 0.8x2.4x2.2m (WxDxH) and floored with concrete. 0.5m of the length was underneath the pod, offering a darker area to the cat (Fig. 4.9). The cat-flap led onto a small wooden shelf (0.17x0.34m), from which ran a ramp to floor level. There was a litter tray at the back of the cage, and a scratching post in the middle of the floor. Unlike the previous two shelters, the corridor from pod to run was not long enough to hide in. The side walls were opaque plastic, so the occupant could not see cats in adjoining pens.

Figure 4.8 Outside corridor.

Figure 4.9 Axehayes outside run

Some of the cats which had been at the shelter a long time were allowed out of their pens, though none of these were studied – around half the 'oldies' had the run of the reception area during the day. This meant that a few cats walked down the central aisle of both Rescue blocks during the day. Further, some cats were allowed out of their runs, and could walk along the outside corridor.

The daily routine was the same for all three blocks. Staff started at 0830, and fed cats and tidied inside pods. This was generally completed by 0945. They then cleaned the runs, which took until 1100. The public was allowed in from 1100-1500, and could go into any of the pods they wished. The cats were fed again at 1530, and staff left at 1630. Most of the staff were part time and worked until 1130. The same staff did generally cover the same areas of the shelter, and the morning cleaning was relatively leisurely compared to the other shelters, allowing a few minutes with some of the cats.

4.2.3 Differences between the shelters

General

Bath was overall a busier shelter than the other two, possibly due to being studied earlier in the year, (i.e. not as close to Christmas as the other two shelters). There were more people inside the shelter, and walking past the outdoor runs. The outdoor runs were more exposed to the public, as there was no outside corridor. There were also dogs either side of the cattery. Though out of sight, they barked often, which appeared to be aversive to many of the cats. Bath generally had a quicker throughput of cats than the other shelters, so cats had generally been at the shelter for less time than those in NCC and Axehayes.

NCC was the largest shelter, and was generally quiet. The corridors were very wide, so cats may have felt less threatened by cats opposite.

Axehayes was quieter than the other two, and had very few visitors. This was possibly due to being studied closest to Christmas, which is usually a quiet time for shelters.

Pod layout

At the NCC, the entire floor was covered with Vetbed, so flooring material did not affect the cat's choice of where to position itself in the pod. The pods had underfloor heating, which crept up the walls so that most cats rested by one or two of the walls.

Bath had blankets in one corner/half of the pod. Though this area was large, cats had to come forward off it to be right next to the door glass. This may have predisposed cats to being in the back corner where the box was later to go.

Axehayes had blankets and heatpads in the beds only. This encouraged the cats to spend most of their time on the one soft and warm part of the pod. Since this would have biased some of the behavioural observations, the night before the study started the author placed a piece of Vetbed on the floor of the pod covering one side at the back and towards the front, so cats were able to be on comfortable material both in the bed and out of it, at the back of the cage and at the front. Starting on day 1 of the study, the heatpads were turned off at 1000 in the morning, and turned on again before the author left the shelter in the evening.

Stressed cats

Bath gave 'igloos' only to very nervous cats. These are roofed cat beds which offer a high degree of 'enclosedness' and can effectively block line of sight both to and from the cat if the cat desires it (Fig. 4.10). One cat which would otherwise have been in the study was omitted as it had an igloo.

At NCC, quite a few cats were given igloos to hide in if not coping. These cats were not included in the study: if the igloo was removed on the first day the author arrived at the shelter (Wednesday, day 0), the first day would be a deprivation day, not a baseline. If the igloo was left with the cat, the difference between baseline and box would have been far less, and boxes may have even been a deprivation as the boxes were less enclosed than some igloos. Only one cat with an igloo was used in the study, and she spent all the day outside and was never observed inside the igloo.

At Axehayes, only one cat in homing (Popsi) had an igloo, and it was a relatively open one, with the open side orientated towards the door. Since the cat did not appear to use the igloo for hiding, she was retained in the final dataset.

Figure 4.10 A typical 'igloo', sometimes given to stressed cats.

Temperature

Despite heating systems, the temperature inside shelters did vary somewhat. This may have altered the cats' preference for being in their bed/box or in the open, so major variations are noted here. CSS especially may have been affected by the cold - Kessler and Turner (1997) noted a minimum of 15°C for the Cat Stress Score, below which cats do not adopt relaxed postures. Accurate CSSs can be taken most of the time for lower temperatures as the CSS frequently depends on other factors which are unaffected by temperature, such as pupil dilation and ear orientation. The cases where CSS may have been affected by cold were noted and checked in the statistical analysis.

Bath: the temperature was 16°C on days 1 and 2, but rose to 20°C on days 3 and 4, as the heating was increased. Since the temperature started off at 16°C, this should not have affected the CSS much, though it later appeared to have done so (Section 4.3.2).
NCC: All blocks had air-conditioning which kept the temperature at 16°C. The heating was turned up on day 2 to 21°C in both homing blocks, though it stayed at 16°C in prehoming. As mentioned earlier, changing from 16°C upwards should not

have had a large effect on posture. More importantly, the air-conditioning broke down on day 3 in both homing blocks, so the temperature in left and right homing fell to 14°C (prehoming remained at 16°C). Right homing was repaired on the morning of day 4 and rose to 16°C, but left homing remained at 13°C. These changes to below 15°C may have caused cats to seek out warmth (from the walls, as the underfloor heating crept up the sides, or by curling up tight), so this was compensated for when assessing CSS.

Axehayes: The heating kept pod interiors at a constant 15 or 16°C for the first three days, but when it broke down on the morning of day 4, the temperature dropped to 12°C, so the day's first scan may have been affected by cold. The temperature was back to normal by the afternoon.

Public

Bath was the busiest shelter, with around 15 people on Thursday and Friday (days 1 and 2), and 40 or so on Saturday and Sunday (days 3 and 4).

NCC: On Thursday and Friday there were around 20 people, in 5 groups. Saturday and Sunday were busy with some people in most blocks most of the time – around 60 people each day.

Axehayes: Thursday and Friday were very quiet – 4 or 5 people on both days. Saturday had no people at all, Sunday around 10. Saturday being very quiet may have had an effect, confounded with day 3 of the study.

Outdoor cats

Some cats in Chapter 3 seemed to spend all of the day in the outside runs. No cats at Bath or Axehayes did this, but 5 NCC cats were resting outside on both baseline scans and the baseline approach test. Of these cats (Kloda, Drusilla, Sophie, Pepper and Maisy), Sophie, Pepper and Maisy had a blanket on one of the outside shelves (the only place they were observed resting). Staff had put the blankets there because the cats routinely spent all of the day outside. It seemed unlikely that removing the blankets would make them go inside, and would clearly reduce their welfare, so the blankets were left in place.

It was of interest whether any of these cats went inside overnight, and would realise the box was there. Since observations during the study might alter the next day's results, on Sunday evening (day 4) the author went back in at 8pm, as quietly as he could. All of the cats were inside at the time (though many ran out again as soon as he came near) aside from Sophie, who was observed inside the pod during box days in any case. Since all of these cats would therefore have had knowledge of the boxes, they were left in the analysis.

4.2.4 Protocol

Cats in each shelter were observed from Thursday to Sunday (Table 4.2). Thursday (day 1) was the 'baseline' day, and cats were observed with their normal pod furniture. Friday and Saturday (days 2 and 3) were 'box' days. A box was placed in each cat's pod in the corner, on the opposite side to the cat flap. In Bath and Axehayes, this was where the existing blanket or bed was. The box was introduced after afternoon feeding on day 1, so the cats had around 17 hours to familiarise themselves with the box before measurements with it were taken on day 2. The box was left in position on day 3 (second box day), and taken away on the morning of Sunday (day 4) the 'deprivation' day. The box was removed 45 minutes before the first observations were taken. This was chosen to allow the cats time to settle down from the disturbance caused by taking the box out of the cage, but was close enough to removal so that any changes in behaviour caused by the box not being present would be noticeable.

Table 4.2 Experimental protocol

Day 1	Day 2	Day 3	Day 4
Thursday	Friday	Saturday	Sunday
'Baseline'	'Box1'	'Box2'	'Deprivation'
No box	Box	Box	No box
Cages left as normal for day's observations. Box added after afternoon feeding.			Box removed before morning scan.

The box used was redesigned from Experiment 2. The box was inspired by the 'Hide and Perch' box used by the British Columbia Society for the Protection of Animals (BCSPCA). Instead of having one open side, one long side had a large hole for the cat to get in / out of, and one short side had a smaller hole so the cat could see out, and so the public could see the cat (Figs 4.11 - 4.14).

The box was double ply cardboard, supplied flatpacked (Transatlantic Plastics Ltd, Southampton, UK). The boxes were glued together with non-toxic PVA glue and parcel tape was used to secure the edges. The roof was reinforced with a rectangle of cardboard the same size as the roof, similarly glued and taped. The roof was then covered with Vetbed (National Veterinary Services, Stoke-on-Trent, UK). The finished box was unfloored and sat directly on top of the Vetbed / blanket in one corner of the pod. This enabled staff to lift the box out and gain access to the cat easily. The box was strong enough that a cat could sit on top of it.

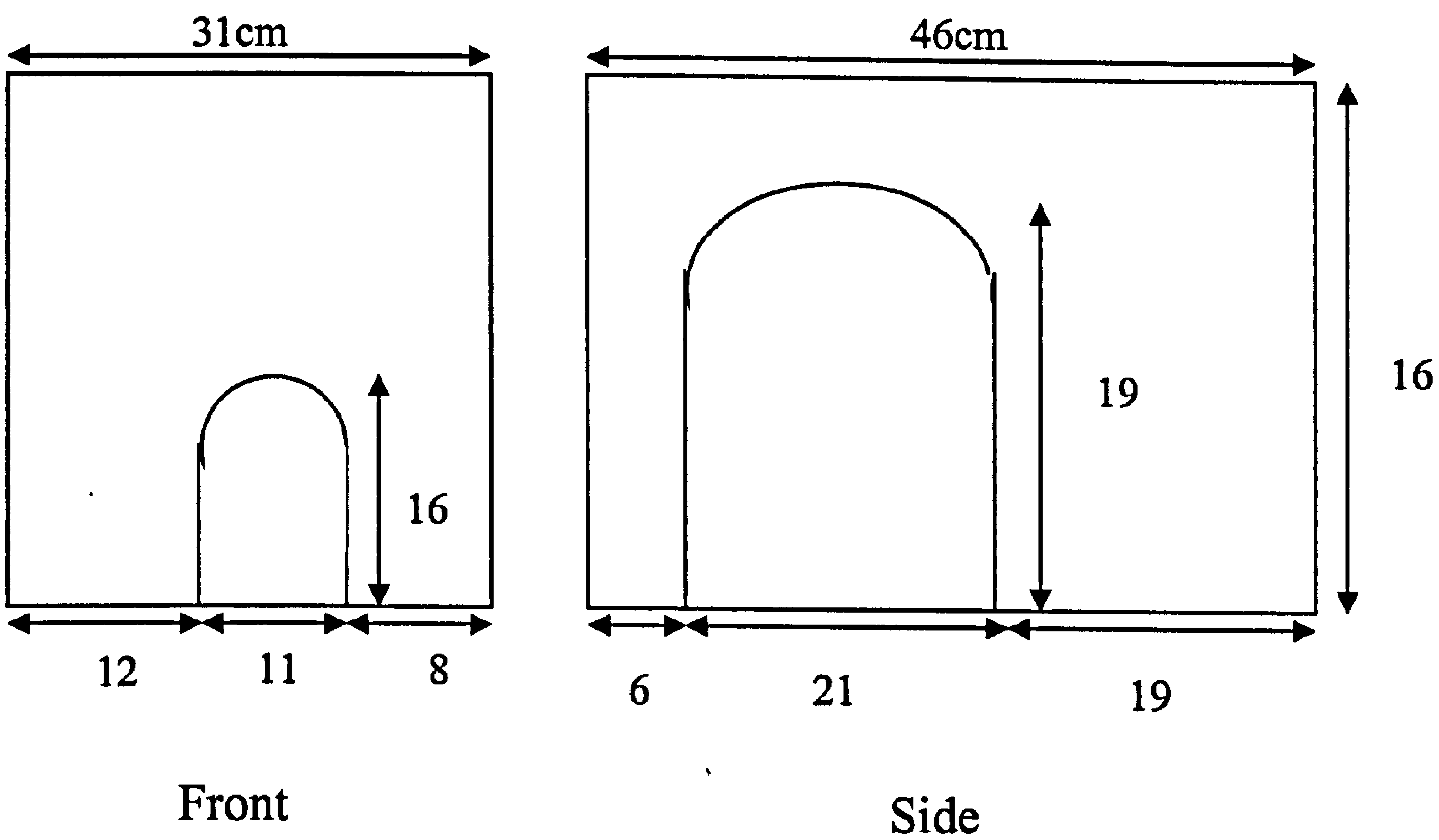


Fig 4.11 Chapter 4 box. All measurements in centimetres. Hidden sides are plain (no holes); no floor to box.

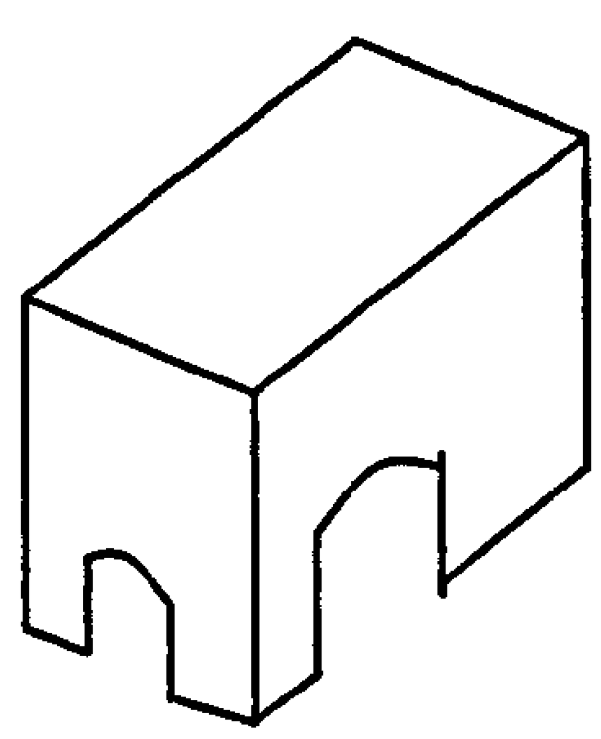


Fig 4.12 Chapter 4 box.

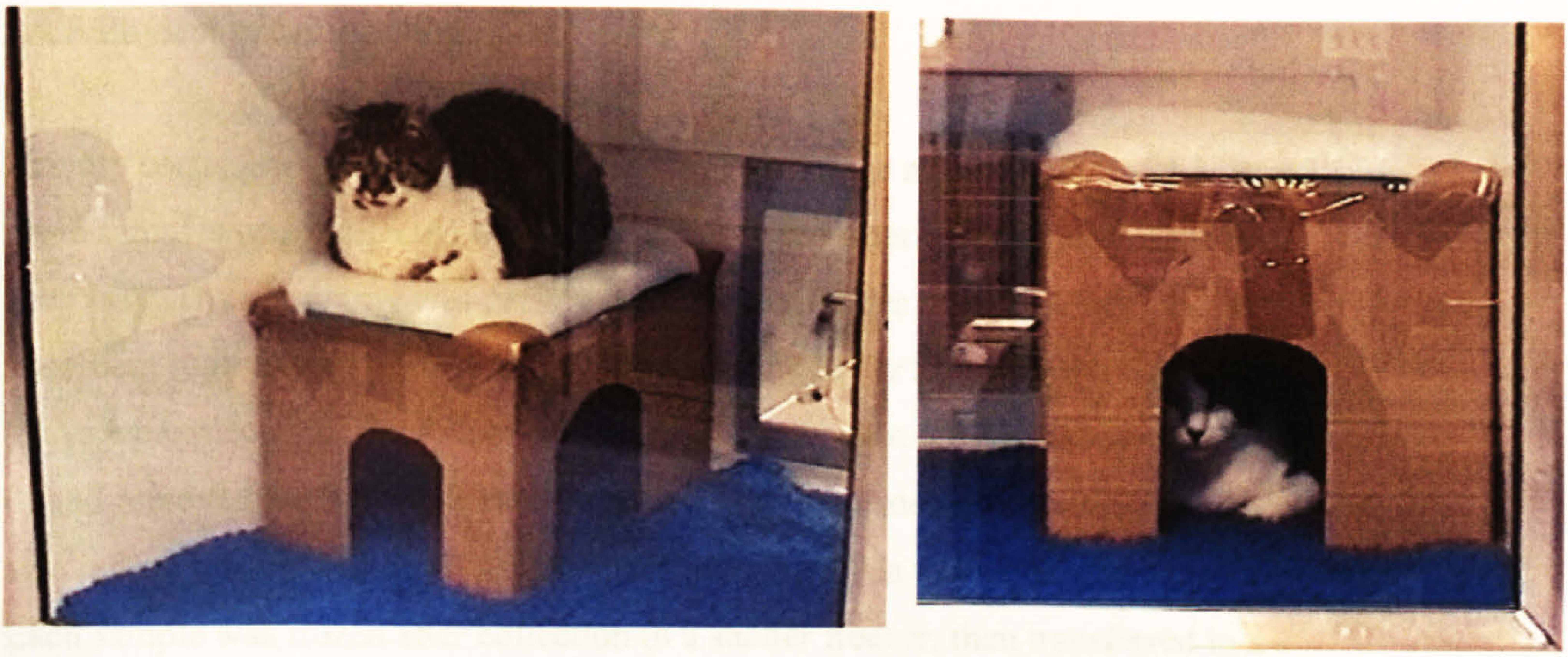


Fig 4.13 NCC pod with box, (Left) cat using the platform (Right) cat looking out of front hole

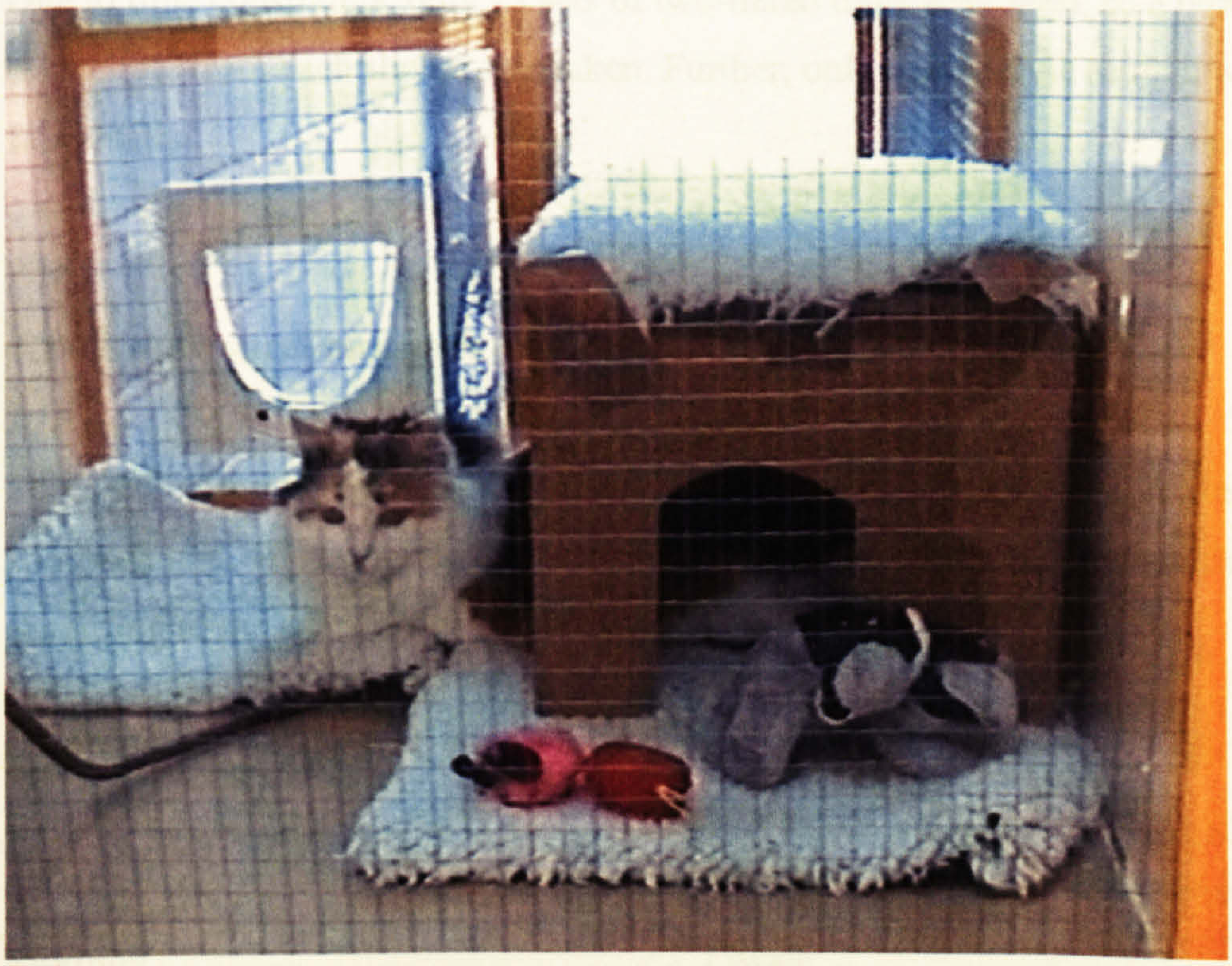


Fig. 4.14 Axehayes pod with box, cat looking out of side hole

4.2.5 Physiological measures

Urinary cortisol was measured using the same technique as Chapters 2 and 3. Briefly, urine samples were collected by leaving a two tiered litter tray in place of the usual litter tray. The upper tray was perforated with small holes and contained plastic non-absorbent litter so that urine drained through to the lower tray where it was collected. Trays were placed in cages shortly before the shelter shut on Wednesday evening (day 0) and removed the following morning. This gave baseline urine samples. Trays were also left on the night of day 3 to give urine samples when the boxes were available. Each sample was frozen after collection in a shelter freezer, then transferred to a -20°C freezer for long-term storage. Samples were analysed for cortisol and creatinine by RIA by Axiom Veterinary Laboratories (Teignmouth, UK).

Due to time constraints, only 13 sets of two-tiered trays were used, so a representative subsample from each shelter was taken. Further, only the baseline sample was taken from Bath.

4.2.6 Behavioural measures

Three sets of behavioural measurements were taken: maintenance, scans and an approach test. They were conducted for each cat on all four experimental days according to the schedule (Table 4.3).

Table 4.3 Daily schedule

Time	*Test*
Early morning	Maintenance
Morning	Scan
Early afternoon	Approach test
Afternoon	Scan
Late afternoon	Maintenance

Maintenance

For each day (starting at around 0900 until the next morning), feeding, urination and whether the cat upset its cage were recorded (Table 4.4). Whether the behaviour took place during the day, or did not take place in the day was recorded. Note that this latter category includes both 'performed overnight' and 'not performed', as the author was unable to arrive at each shelter early enough every day to check if a behaviour was performed overnight or not. Since there were very few instances of 'not performed' it seemed reasonable to merge it with 'performed overnight'. Defaecation was not recorded partly because staff sometimes removed faeces during the day, and partly due to time constraints.

Table 4.4 Maintenance measures recorded

Measure	*Definition*
Feed	Some food missing from food bowl / food bowl taken away because empty
Urinate	Wet litter in tray
Upset cage	Evidence of aggression / excessive escape behaviour directed at cage furniture: box being torn, blankets pulled around, food and water bowls overturned.

Approach test

An approach test was used to record the cats' response to humans, derived from Kessler and Turner (1997), and Garman (2002). The test consisted of two phases. In phase 1, the author approached the pod from the front, put one hand up against the glass and greeted the cat with 'hello cat'. He remained for 30 seconds, saying 'hello cat' at 10 and 20 seconds. After the 30 seconds, he opened the cage door and put his hand just within the cage, again saying 'hello cat' upon entry, and at 40 and 50 seconds. To speed up conducting over 50 approach tests in the large catteries, he tried not to touch the cat (as this would require him to wash his hands). Sniffing was allowed, but he withdrew his hand if the cat tried to rub against it, if necessary withdrawing and closing the door. If so he kept his hand against the glass until the minute was up. Measures taken are below (Table 4.5).

Table 4.5 Approach test measures

Measure	*Definition*
Appclose	Behaviour during first 30s when cage door closed: 1 = Approach 2 = Withdraw 3 = Neither If both approach and withdraw occur, coded by first occurrence.
Appopen	Behaviour during second 30 when cage door open, as above.
LatencyApp	Latency to approach in seconds (1-60). If none, scored as 61s.
LatencyWith	Latency to withdraw, as above.
CSSstart	CSS of the cat at the very start of the test
CSSfin	CSS at the end of the test
CSSupdown	Whether CSSfin was higher than CSSstart. (In some approach tests where the CSS stayed the same, it was nonetheless possible to note an incremental increase or decrease in CSS.)
Friendliness	Friendliness, measured on a 1-5 scale: 1 = very friendly 2 = friendly 3 = neither friendly not unfriendly 4 = aggressive / fearful 5 = very fearful For more details see below.
Friendliness '1'	"very friendly": cat is orientated towards, and shows friendly behaviours to the observer for the duration of the minute. Shows no fearful[2] or aggressive[3] behaviours.
'2'	"quite friendly": cat is orientated to, and shows friendly behaviours to, the observer during the minute, but not continuously – some behaviours are not directed towards the observer, e.g. investigating other parts of the cage, looking away from the observer, grooming. No, or few, aggressive behaviours. Some friendly cats which were nervous fall into this category as they are also focusing on their environment.
'3'	"neither": cat shows no friendly or aggressive behaviours. May or may not be orientated towards the observer. Typically, a cat which either does not approach, or ignores the observer. The occasional cat which shows a mix of friendly and unfriendly behaviours was put into this category.
'4'	"aggressive / fearful": cat is orientated to the observer during the minute. None, or few, friendly behaviours. May be some unfriendly behaviours. Will show some fearful behaviours. Typically, a cat which looks nervous but does not flee or react aggressively

'5'	"very fearful": cat is orientated to the observer the entire minute. No friendly behaviours. Will show unfriendly or fearful behaviours. Typically, a cat which withdraws into the other part of the accommodation (pod or run) and/or reacts aggressively upon approach.

Notes:
[1] "friendly" behaviours – Tail up and may be vibrating, rubbing against observer or cage furniture, greeting vocalisations, ears forward, may be purring.
[2] "fearful" behaviours – Ears back, staring, lowered posture, postural or body withdrawal. Tail close to body.
[3] "aggressive" behaviours – Growling, yowling, hissing, may strike out with paw, staring. Nearly always combined with fearful behaviours when performed towards observer.

The 'friendliness' test scoring was similar to Chapter 3 scorings though with Chapter 3's 'quite friendly' and 'slightly friendly' categories merged into '2 = friendly'. The approach test was carried out in between the two scans, at least half an hour after the finish of the first scan. At the NCC and Axehayes, the author sometimes had to stagger the tests together, i.e. if the order of the approach test was block A, then B and then C, he started the afternoon scan in block A as soon as he had been away from block A for half an hour.

Scans

Scans were performed twice a day. Each scan involved the author standing in front of a pod recording the position and behaviour of the occupant, ending in scoring the CSS. The various measures and categories for scoring are listed below (Table 4.6). The CSS was recorded as per Chapter 3, though in response to slight concerns of the author about the CSS, 'tense sleep' was changed to being scored as 3.5 unless obviously very tense.

The order of cats scanned was randomised for each scan. Each block was scanned separately. Before staring the scans in each block, the author walked up and down its length once so that all the cats realised he was there. If the cat due to be scanned was directly opposite or beside the previous cat scanned, it was left until the other cats in the block had been scanned. Cats which were grooming or had had members of the public close to them within the last few minutes were also left until the end. Since some cats continued to have people very near to them, or groomed for some time, he frequently had to return to the same block two or three times to successfully scan all the inhabitants.

The first scan each day was started 45 minutes after the shelter opened, and lasted until all cats had been observed (usually a little less than an hour). The second scan was towards the end of the day, at least half an hour after the approach test. It was timed such that it should finish half an hour before the shelter closed (to avoid excitement due to feeding, which commenced 15 minutes or so after closure).

Table 4.6 Scan measures recorded.

Measure	*Definition*
InOut	In pod = 1 Out in run = 2
Hide	Not hiding = 1 Hiding (or trying to) = 2
Face	Face front (towards observer) = 1 Face side = 2 Face back = 3 Face back, looking outside = 4 Other = 5
Behaviour	Active = 1 Resting eyes open = 2 Resting eyes closed = 3 Other = 5
Position: Inside pod:	 Front of cage = 1 Back of cage, not in box / bed area = 2 Back of cage, in box / bed = 3 On top of box (where applicable) = 4 Other = 5
Out in run:	Front of cage = 1 Back of cage = 2 Under ramp = 3 On shelf = 4 Other = 5
Exposed: No box day (bed / blanket present)	 Out in open = 1 In bed, head visible = 2 In bed, head not visible = 3 Behind bed = 4 Other = 5
Box day	Out in open = 1 In box, head out = 2 In box, head in, visible = 3 In box, head not visible = 4 Other = 5
Hfbf	Head closest to door = 1 Body closest to door = 2
Posture	Standing / active = 1 Sitting = 2 Lying ventral = 3 Curled up = 4 Lying on side = 5 Lying ventral, on all four paws = 6

4.2.7 Analysis

Eight cats were not available for all 4 days of the study – six were rehomed halfway through, and two underwent 24 hour starvation for medical tests. Since all cats were however fit and well before these events, their earlier days' data was kept in the dataset for analysis. Day 1 and 2 had N=89, which fell to N=81 by day 4.

Demography

To check if any of the demographic variables differed between shelters, Kruskal-Wallis tests (non-parametric test for independent subjects) were performed for each variable to look for differences between the three shelters. Because Bath's small N may reduce the power of the K-W test, the difference between NCC and Axehayes only was also tested, with Mann-Whitney U-tests.

For all variables (sex, stray, sex*stray, breed, age, liking other cats and size), the K-W and Mann-Whitney tests were NS at $p > 0.1$, apart from 'size' at $p = .09$. It was concluded that there seemed to be no difference between subjects at the different shelters large enough to affect the results.

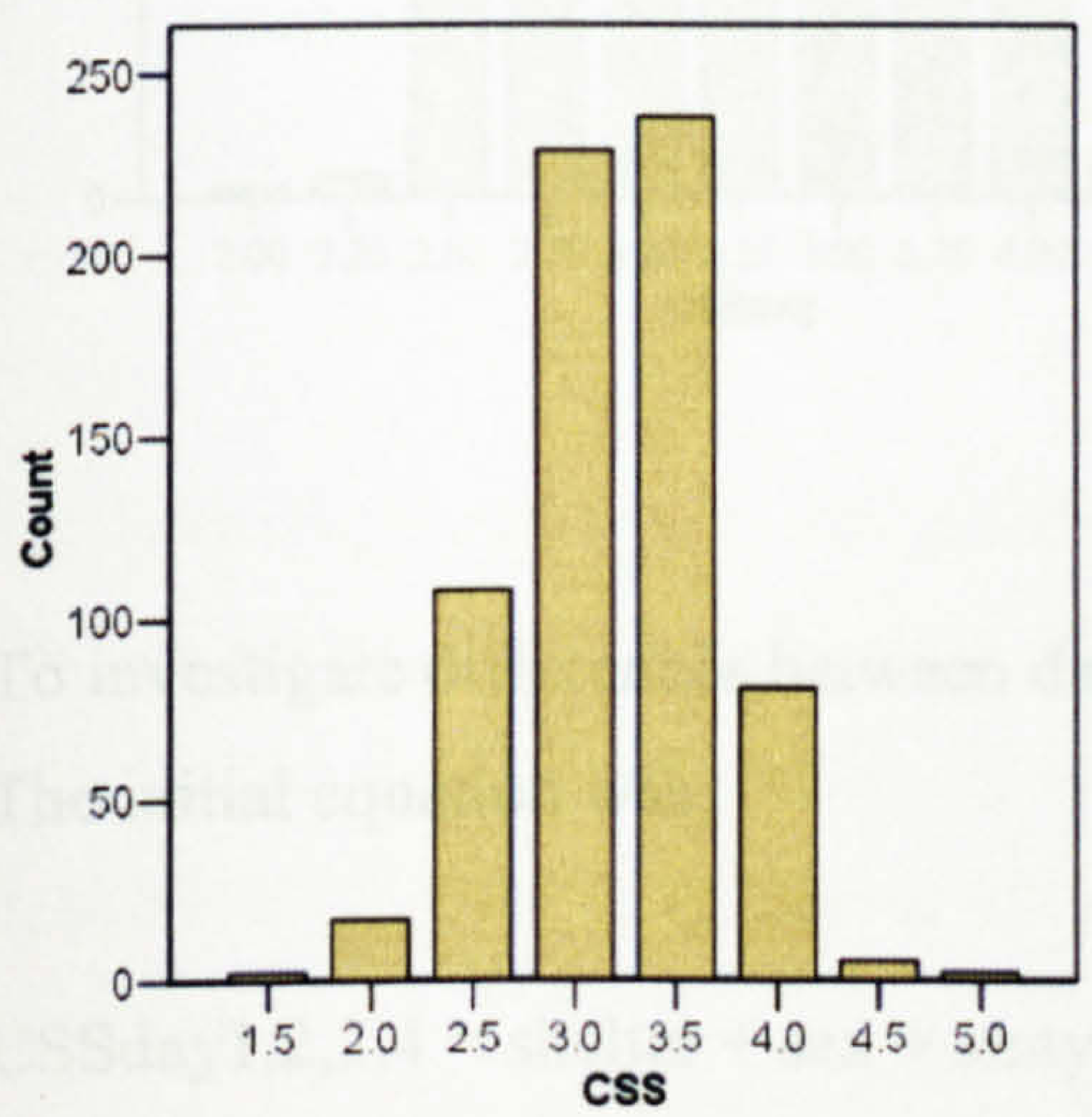
Maintenance

Only one incidence of *upset cage* was recorded, so no further analysis was performed on this variable. To look for differences in *feed* and *urinate* between shelters, Chi-squared tests were conducted for each day separately. This was done to compare data from all 3 shelters, and also to compare NCC and Axehayes (as Bath cats were such a small part of the final dataset). To look for changes in *feed* and *urinate* over the 4 days, Friedman's test (non-parametric test for related samples) was used.

CSS

CSS using pooled data from all days and all scans resembled a normal curve (Fig. 4.15), with the average between CSS 3 and 3.5, suggesting that most cats are weakly tense or between weakly tense and very tense.

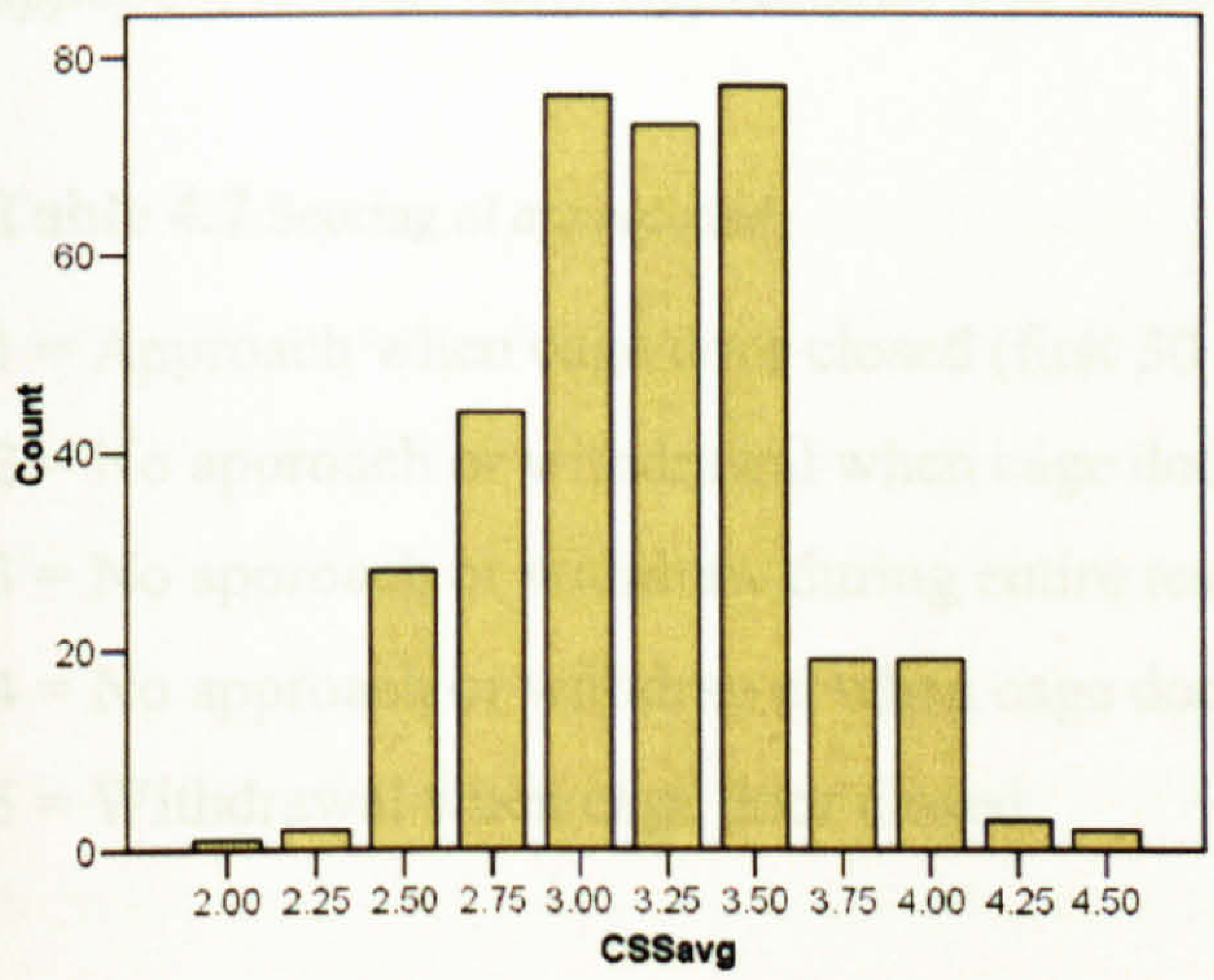
Fig. 4.15 The number of scans showing each class of CSS (data from all 682 scans on the 89 cats)



A Wilcoxon signed ranks test was used to compare *CSSmorn* and *CSSaft* from each cat and day (N=338). There was no significant difference ($p > 0.1$). Separate Wilcoxon's tests for each day's data were also all NS at $p > 0.1$. Since the two populations were not significantly different, the median for each day was used to give each cat's CSS datum for that day (*CSSavg*) (Fig. 4.16). *CSSavg* approximated a normal curve, and with 11 categories, approximates a continuous variable. Previous studies (e.g. Kessler and Turner 1999b) have also treated the CSS as continuous data.

CSSavg skewness and kurtosis were both low (.13 and .26 respectively). Kolmogorov-Smirnov tests of normality were significant at $p<.001$ though this is quite common for large datasets due to the high power of the test and should not be of concern in itself. Transformation was not indicated as necessary by the data.

Figure 4.16 The number of cats showing each class of *CSSavg* (pooled data from all 4 days)



To investigate differences between days, repeated measures GLM was used. The initial equation was:

CSSday1,2,3,4 = shelter + sex + stray + sex*stray + $age_{(covar)}$

Size, *othercats* and *urban* were not added as a sizeable portion of the dataset did not have this information available. Terms which did not add anything significant to the model (as judged by significance of the multivariate, within-subjects and between-subjects tests) were removed, and the model retested.

Box's test of equality of covariance matrices, Mauchley's test of sphericity and Levene's test of equality of error variances and Lack of fit tests were all NS unless mentioned in the text.

Approach test

A new variable was created from the existing ones – *apprecoded*. *Apprecoded* was made by merging the results from *appopen* and *appclose* into one variable. The first occurrence of an approach or withdraw only was counted (i.e. if the cat's first movement is an approach in *appclose* then what it does later in *appclose*, or in *appopen*, is irrelevant). *Apprecoded* was scored as per Table 4.7.

Table 4.7 Scoring of *apprecoded*

1 = Approach when cage door closed (first 30 seconds)
2 = No approach or withdrawal when cage door is closed, approach once door open.
3 = No approach or withdraw during entire test
4 = No approach or withdrawal when cage door is closed, withdraw once door is open
5 = Withdrawal when cage door closed

For *latencyapp* and *latencywith*, nearly all data were clustered around three points: the start of the test, when the door was opened, or making no approach or withdrawal. This makes the data very similar to *appclosed* and *appopen*, so *apprecoded* was used instead as it is more informative, allowing approach and withdrawal in the same measure.

Differences between shelters

Chi-squared tests were used to check for differences in *apprecoded* between all 3 shelters, and also between NCC and Axehayes only. Each day's data was analysed separately.

To check for differences between the shelters for *friendliness* and the two CSS variables, K-W tests were conducted for each day, looking for differences between shelters. Mann-Whitney tests were used to look at differences between NCC and Axehayes.

Differences between days

To look at changes in *apprecoded* and *friendliness* over time, Friedman's test for related samples was performed on data from all 4 days. A McNemar-Bowker test was used to look for changes between pairs of days. This tests for changes in responses using the chi-square distribution (SPSS Inc 2003).

Many classes of *apprecoded* appeared to change over the box days. To look at variations in individual classes, Cochran's Q was used (equivalent to a Friedman test on bivariate data) for each class in turn. E.g. for class 1, all other values (2-5) were set to 0, so the test looked at the distribution of class 1 only.

To see if *CSSstart* or *CSSfin* vary across days, Friedman tests were used. McNemar-Bowker tests were performed on *CSSupdown* for pairs of days

Scan

Some scans were difficult to score using only Table 4.6:

Hiding under the blanket (only occurred at NCC). If the cat is hiding, its precise behaviour, posture and facing are not known. However, this behaviour does indicate a high motivation to hide, so it is important to keep in the dataset if possible. Blanket covered cats were treated as follows:

In/Out = (as normal)
Hide = 2 (hidden)
Face = 3 (back, as the cat cannot see out at all).
Behav = 3 (resting eyes closed attentive, as cat cannot see out and is unlikely to be relaxed)
Posn = 5 (blanket)
Exposed = 5 (other)
CSS = 4 (very tense)
Hfbf = not recorded
Posture = not recorded

Sitting in a cat bed (possible at Axehayes only) was classed as *exposed* = 1 (in the open), the same as lying on the blanket with head visible, despite the cat bed. This was done because the cat beds have fairly low sides, and if sitting up the cat is voluntarily making itself far more exposed than it needs to be.

Differences between shelters

To look for differences between shelters, Chi-squared tests were conducted for each variable using data from each of the 8 scans. Differences between all 3 shelters were looked at initially, followed by differences between NCC and Axehayes, as before. This gave 16 tests per variable. Many of the tests had half the cells with expected values of less than 5. The Chi-squared statistic is still fairly robust with up to half the cells less than 5 given a large N, so this was not a huge concern.

Differences between days

To look for changes over the 4 days, Cochran's Q tests were conducted on *InOut*, *Hide* and *hfbf*. *Face* was recoded into looking out to the front or not looking to the front, and then subject to Cochran's Q. With the elimination of the 'other' category (one cat scored 'other' on day 4, that datum was removed from the *behaviour* analysis without replacement), *behaviour* becomes an ordinal variable of 'alertness', and was tested with Friedman tests, as was *exposed* (no cats scored 'other').

Ordinal data (including bivariate categorical data) was then investigated using marginal homogeneity tests to investigate differences between pairs of days. For categorical data (position and posture), pairs of days were then investigated using McNemar - Bowker tests.

Cortisol

CC ratios (mol cortisol:mol creatinine)x10^6 were calculated for all cats which had results from both collection days: 6 from NCC, 7 from Axehayes. The change in CC (baseline minus box CC) was also calculated for each cat.

Differences between shelters

To investigate differences between shelters, independent samples T-tests were conducted for the variables baseline CC, box CC and the change in CC.

Differences between days

To see if CC changed over time, paired samples T-tests were used on baseline and box CC.

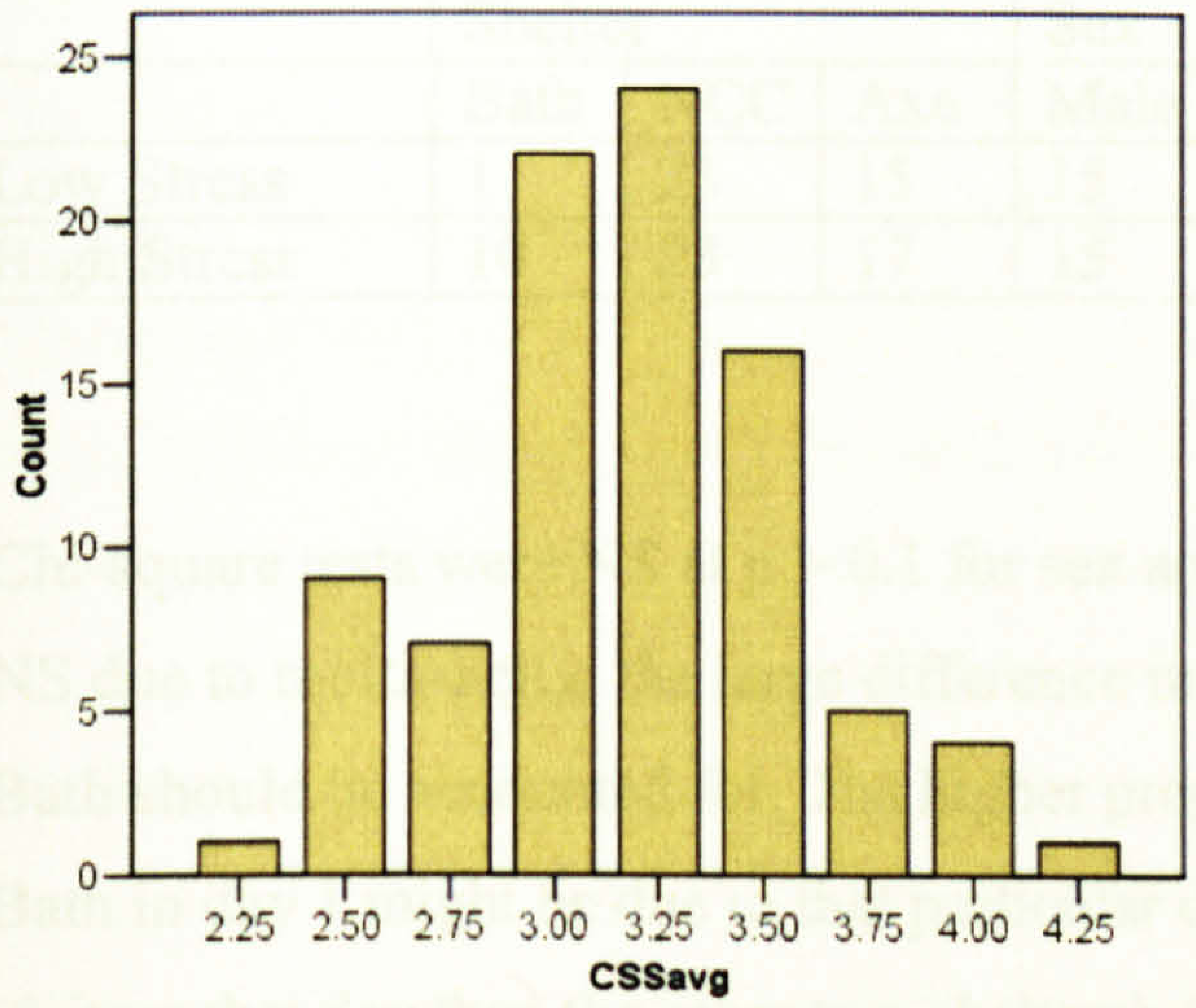
Relations with other variables

Baseline CC was compared to other day 1 variables in a GLM to see if CC was related to any of the other variables studied. Due to the low N there were few degrees of freedom available, so univariate equations were used. For variables with a high number of categories (e.g. *approach*, *friendliness*, *behaviour*, *position* and *exposed*) even univariate equations did not have enough degrees of freedom so these analyses could not be performed. Variables analysed were: *CSSavg*, *inout*, *hide*, *hfBf* and *face* (coded bivariately). Repeated measures GLMs were also conducted to investigate whether changes in CC over time were linked to any of these variables.

Baseline stress

To see if cats which were stressed at baseline showed a different response to the box than cats which were not, each cat was classed as '*high stress*' or '*low stress*' according to day 1 ("baseline") CSS*avg* (Fig. 2.17). From Figure 2.17, the data are evenly split around 3.25. Dividing below 3.25 gave the most even split, which (all else being equal) increases statistical power. Histograms of variables to be tested, clustered by 'high' and 'low' stress suggested that splitting below 3.25 resulted in a greater difference between 'high' and 'low' stress than splitting above.

Figure 4.17 The number of cats showing each class of *CSSavg* on day 1



This gave: 2.25-3.0, N = 39 (*low stress*); 3.25 – 4.25 N = 50 (*high stress*). Plotting demographic variables against *stress*, there was no obvious difference between ages (independent samples T-test, $p > 0.1$), but some between shelter, *sex* and *stray* (Table 2.8).

Table 4.8 Shelter, sex and stray, split by *stress*.

	Shelter			Sex		Stray	
	Bath	NCC	Axe	Male	Female	Owned	Stray
Low Stress	1	23	15	15	24	31	6
High Stress	10	23	17	15	34	31	15

Chi-square tests were NS at $p > 0.1$ for sex and stray, and $p = .045$ for shelter. Though NS due to multiplicity, the large difference in N between *low*- and *high stress* cats at Bath should be accounted for. The higher proportion of cats in the high stress group in Bath in day 1 might be due to that particular day being more stressful (Bath had more visitors that day than the other two shelters). It might instead be that Bath cats are generally more stressed, possibly due to having more visitors, having people passing by the outside run more often, or because the cats had generally been at the shelter for less time than cats at the other two shelters. Since measured CSS is a mix of environmental and individual (personality) factors in any case, these cats were left in the dataset.

This new variable, *stress*, was used to investigate whether cats with high baseline stress responded differently to inclusion of the box than cats with a low baseline stress.

Baseline stress and CSS

Stress was added as a categorical factor (x-variable) to the starting equation in section 4.2.7 and analysed using the same method. Data from CSS 1 was not included as it had been used to derive *stress*.

$$\text{CSSday2,3,4} = \text{shelter} + \text{sex} + \text{stray} + \text{sex*stray} + \text{age}_{(\text{covar})} + \text{stress}$$

Baseline stress and approach test

Apprecoded, *friendliness* and *CSSupdown* were analysed using baseline CSS. Each day was analysed for differences between *low stress* and *high stress* groups – *apprecoded* with Chi-sq tests, *friendliness* and *CSSupdown* with Mann-Whitney U tests. Individual classes were also analysed using Cochran's Q for each class in turn.

Baseline stress and scan test

Of the scan variables, only four (*hfbf*, *face*, *exposed* and *position*) had significant differences between days (Section 4.3.3). Of these, *hfbf* had no difference other than on day 1, and *face* was confounded with differences between shelters, so only *exposed* and *position* were studied to look at the effect of *stress*. Each scan for these two variables was analysed for differences between *high stress* and *low stress* groups – *position* with Chi-square, and *exposed* (recoded as ordinal data, see Section 4.2.7) with Mann-Whitney U tests. Individual classes were also analysed using Cochran's Q for each class in turn.

Baseline stress and cortisol

Independent sample T-tests were conducted between *low stress* and *high stress* cats for baseline CC, box CC, and the change in CC. GLMs were performed as per Section 4.2.7. The initial equation was:

CCbaseline, box = stress + shelter + stress*shelter

4.3 Results

4.3.1 Maintenance

Feed: Of 331 valid observations, 311 were of eating during the day, with only 20 of eating overnight (there were no cases of cats not eating). This may be expected in cats which have become used to shelter life.

Urinate: Of 324 valid observations, 163 were of urination during the day and 161 overnight or not at all. Although stressed cats are frequently inhibited from urinating during the day, this is not a sign of stress *per se*, as shelter cats typically urinate infrequently (pers obs), which may be due to aspects of life in a shelter that affect even cats which have adapted well to the shelter.

Differences between shelters

The results from Chi-squared tests for differences in *feed* and *urinate* between shelters are below (Table 4.9). No maintenance data was collected from Bath on day 1.

Table 4.9 P-values for Chi-squared analysis of maintenance variables – differences between shelters by day, for all 3 shelters, and for NCC and Axehayes only.

	Feed All 3	Urinate All 3	Feed NCC, Axe	Urinate NCC, Axe
Chi^2 day 1			.230	.022*
Chi^2 day 2	.005**	.776	.018*	.649
Chi^2 day 3	.610	.061	1.00	.638
Chi^2 day 4	1.00	.212	1.00	.473

After Bonferroni correction for the 4 days tested, the only significant result is on day 2, where there was a significant difference in feeding. Bath has the highest proportion of cats not feeding during the day, followed by NCC. On day 1, NCC had a higher proportion of cats not urinating during the day than Axehayes, though this was not significant after correction for multiplicity. Since there is no systematic difference between shelters, results from all 3 shelters were combined. There will always be some variation between shelters due to day-to-day changes in number of people, etc, which will be part of error variation.

Differences between days

The Friedman's test for changes in feeding and urinating over the 4 days had $p = .003$ and $p = .300$ respectively. Although there is no significant change in *urinate* over the 4 days, there is in *feed* (Table 4.10).

Table 4.10 Number of cats not feeding during the day, by shelter and day.

	Total	Bath	NCC	Axehayes
Day 1	7	No data	6	1
Day 2	12	4	8	0
Day 3	1	0	1	0
Day 4	0	0	0	0

Under normal conditions, if there was a change in feeding, one would expect fewer cats to feed during the day at the weekend, as there are more visitors to the shelter. The distribution observed may have been a reaction to the observer, which dropped over time as the cats became used to him. Although non-significant, *urinate* follows a similar trend (Table 4.11). So, there appears to be no effect of the boxes on the proportion of cats urinating, though there may have been an effect of the observer, or of the experiment generally.

Table 4.11 Proportion of cats not urinating during the day (N varies between 78 and 87 per day).

	Proportion not urinating during the day
Day 1	0.54
Day 2	0.52
Day 3	0.49
Day 4	0.41

4.3.2 CSS

The initial model was:

CSSday1,2,3,4 = shelter + sex + stray + sex*stray + $age_{(covar)}$

Age was dropped from the equation as it was NS for multivariate, within-subjects and between-subjects tests. This left the equation:

CSSday1,2,3,4 = shelter + sex + stray + sex*stray

in which all variables were significant, or close to significance, so could not be improved by removing variables (Table 4.12)

Table 4.12 p-values for terms in the GLM equation CSSday1,2,3,4 = shelter + sex + stray + sex*stray. N = 76, * = $p < .05$, ** = $p < .01$.

Variable	Multivariate tests	Within-subjects effects
Day	.001**	<.001**
Day*Shelter	.003**	.001**
Day*Sex	.449	.463
Day*Stray	.195	.188
Day*Sex*Stray	.157	.170

Variable	Between-subs effects
Shelter	.115
Sex	.006**
Stray	.973
Sex*Stray	.001**

So there is a significant change in CSS over time (Within-subjects Day $p < .001$), and this change is different in the three shelters. Both sex and the interaction between sex and stray both have significant between-cat effects. The Lack of Fit tests for this model were significant however, which suggest that the model is not adequately describing the data. To investigate this, graphs of marginal means of CSS were created, split by shelter (Fig.4.18) and sex*stray (Fig. 4.19).

Figure 4.18 Estimated daily marginal means for CSS, split by shelter

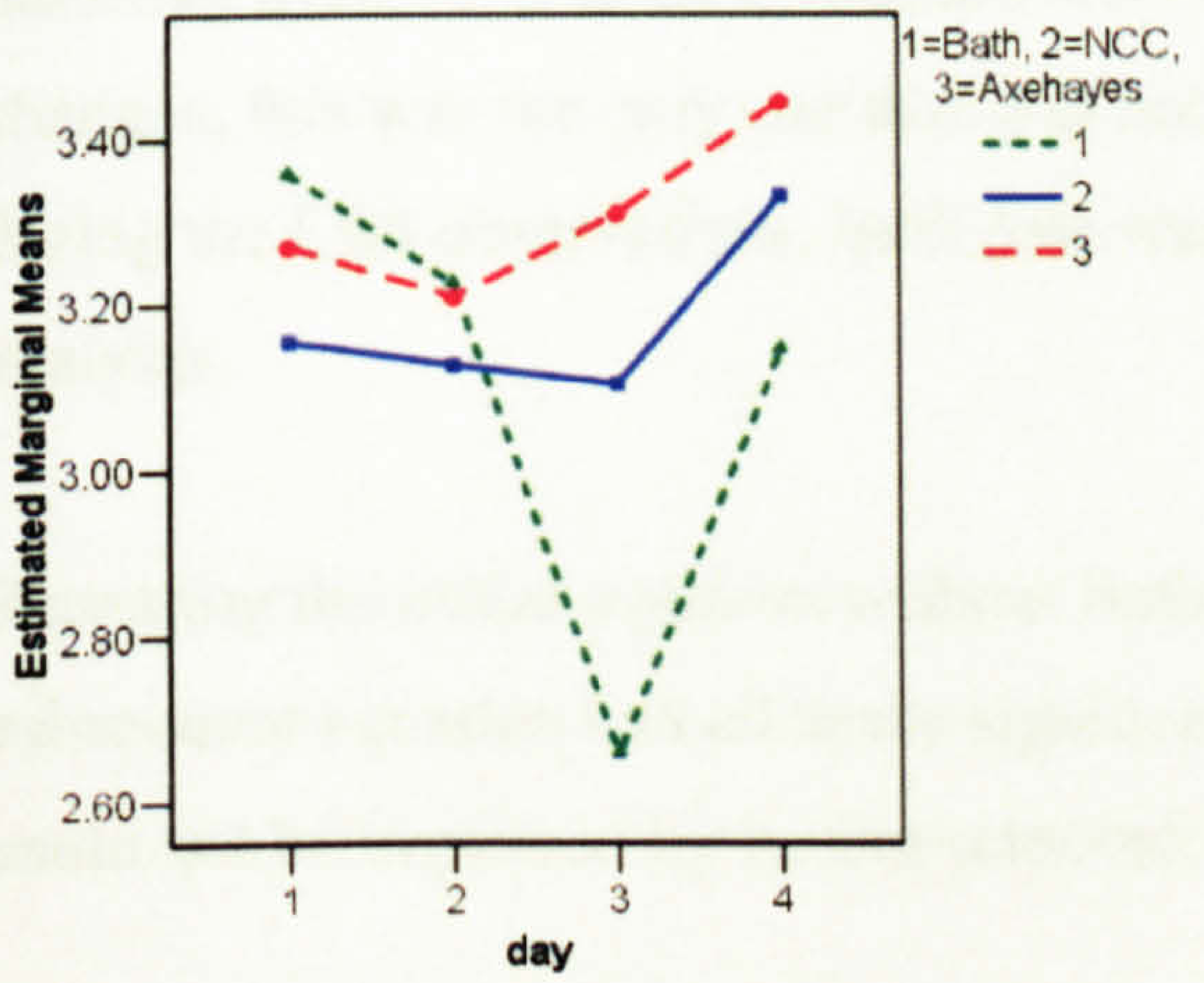
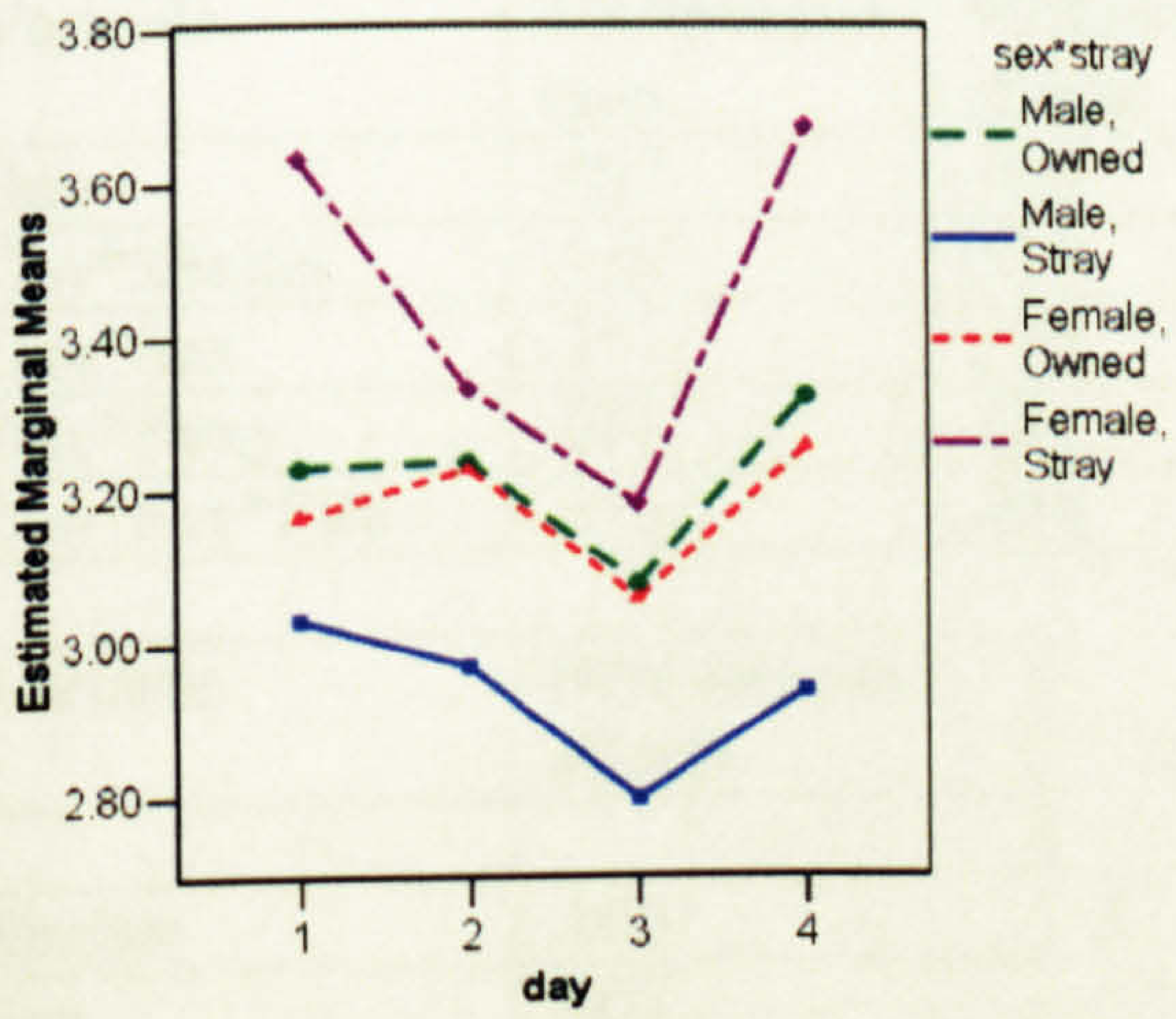


Figure 4.19 Estimated daily marginal means for CSS, split by Sex*Stray



From the graph of estimated means split by sex*stray, it seems as though there is an effect of sex (females having higher CSS), though the effect of stray differs for male and female strays, which is why female has a significant between-subjects effect but stray does not. This also shows the within-subjects effect of stray – strays have more of a dip on days 2 and 3 than owned cats, but the high CSS of female strays cancels out the low CSS of male strays in the between-subjects test.

From the graph split by shelter, the data from Bath is very different from other shelters on day 3, which has far lower CSS. The heating was turned up in Bath on Saturday from 16^{0}C to 20^{0}C (section 4.2.3). Although other shelters had temperature changes, this was the only one that was not explicitly compensated for by the author during the CSS observations. Bath data was therefore removed from further CSS analysis.

Repeating the initial equation without Bath data again suggested removing 'Age'. The subsequent equation had all terms significant or close to significance (Table 4.13), so could not be improved by further removal:

CSSday1,2,3,4 = shelter + sex + stray + sex*stray

Table 4.13 p-values for terms in the GLM equation CSSday1,2,3,4 = shelter + sex + stray + sex*stray, no Bath data. N = 69

Variable	Multivariate tests	Within-subjects effects
Day	.007	.005
Day*Shelter	.536	.519
Day*Sex	.371	.375
Day*Stray	.051	.038
Day*Sex*Stray	.376	.408

Variable	Between-subs effects
Shelter	.105
Sex	.007
Stray	.718
Sex*Stray	.002

Graphs of marginal means of CSS are below, unsplit and split by shelter, sex, stray and sex*stray (Figs. 4.20, 4.21, 4.22, 4.23 and 4.24). Lack of fit tests were again significant, so the model was not explaining the data well. Adding 'size' to the equation (with the removal of Bath, nearly all cats had data for this variable) was NS at $p > 0.1$, as were 'breed' and 'other cats'. Having run out of variables to test, this issue could not be addressed further.

Figure 4.20 Estimated daily marginal means for CSS, averaged for Axehayes and NCC only

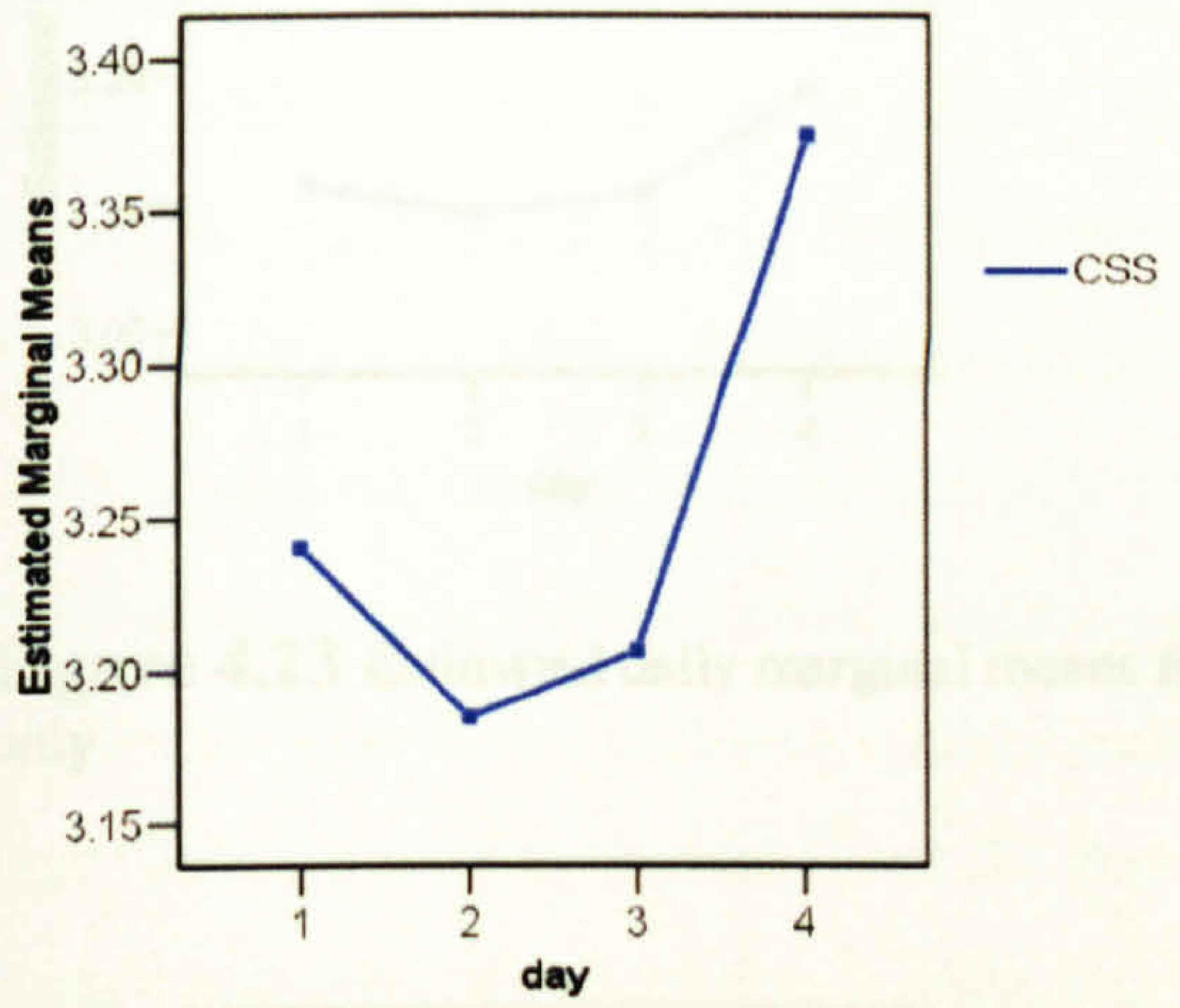


Figure 4.21 Estimated daily marginal means for CSS, split by shelter

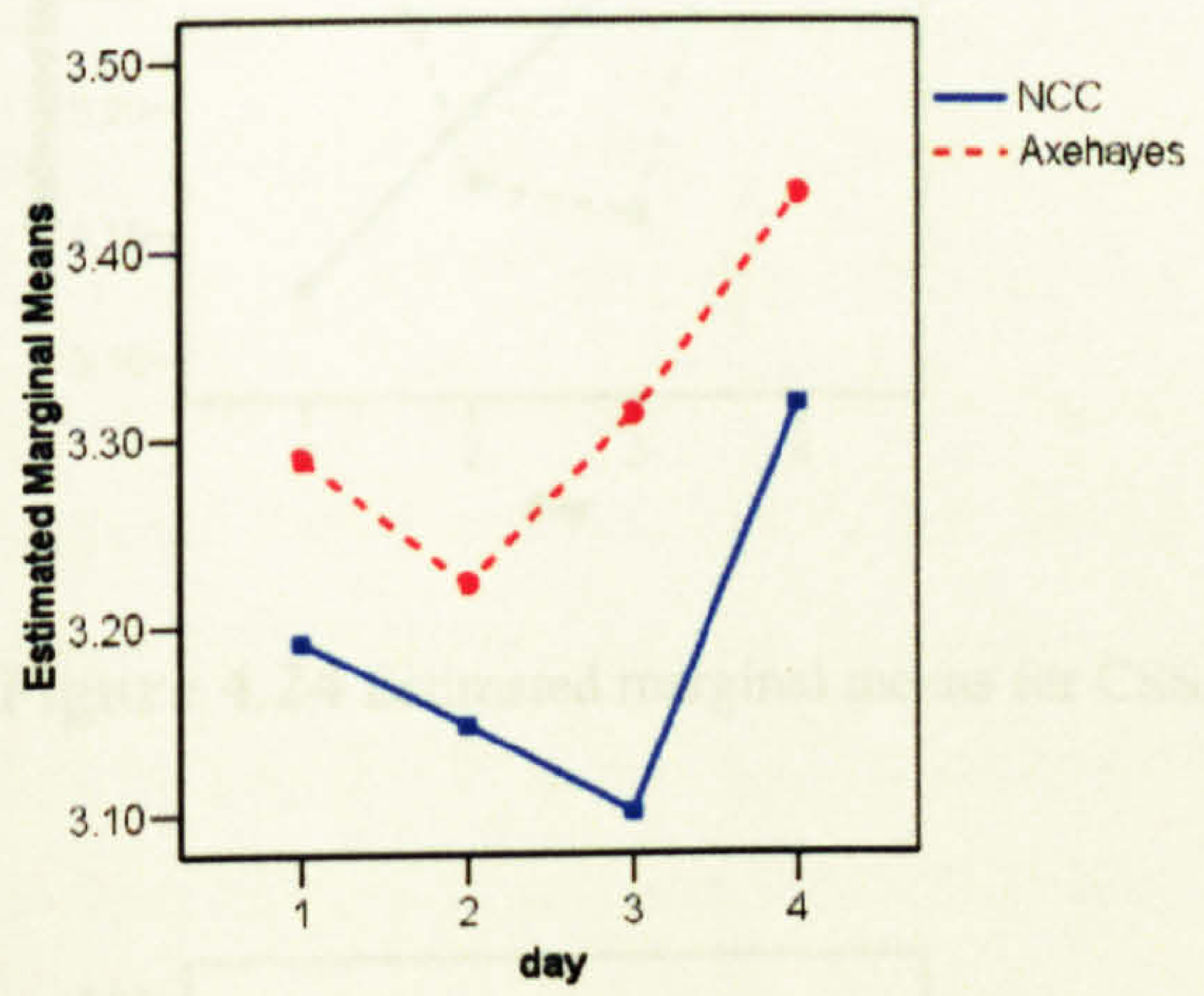


Figure 4.22 Estimated daily marginal means for CSS, split by sex, Axehayes and NCC only

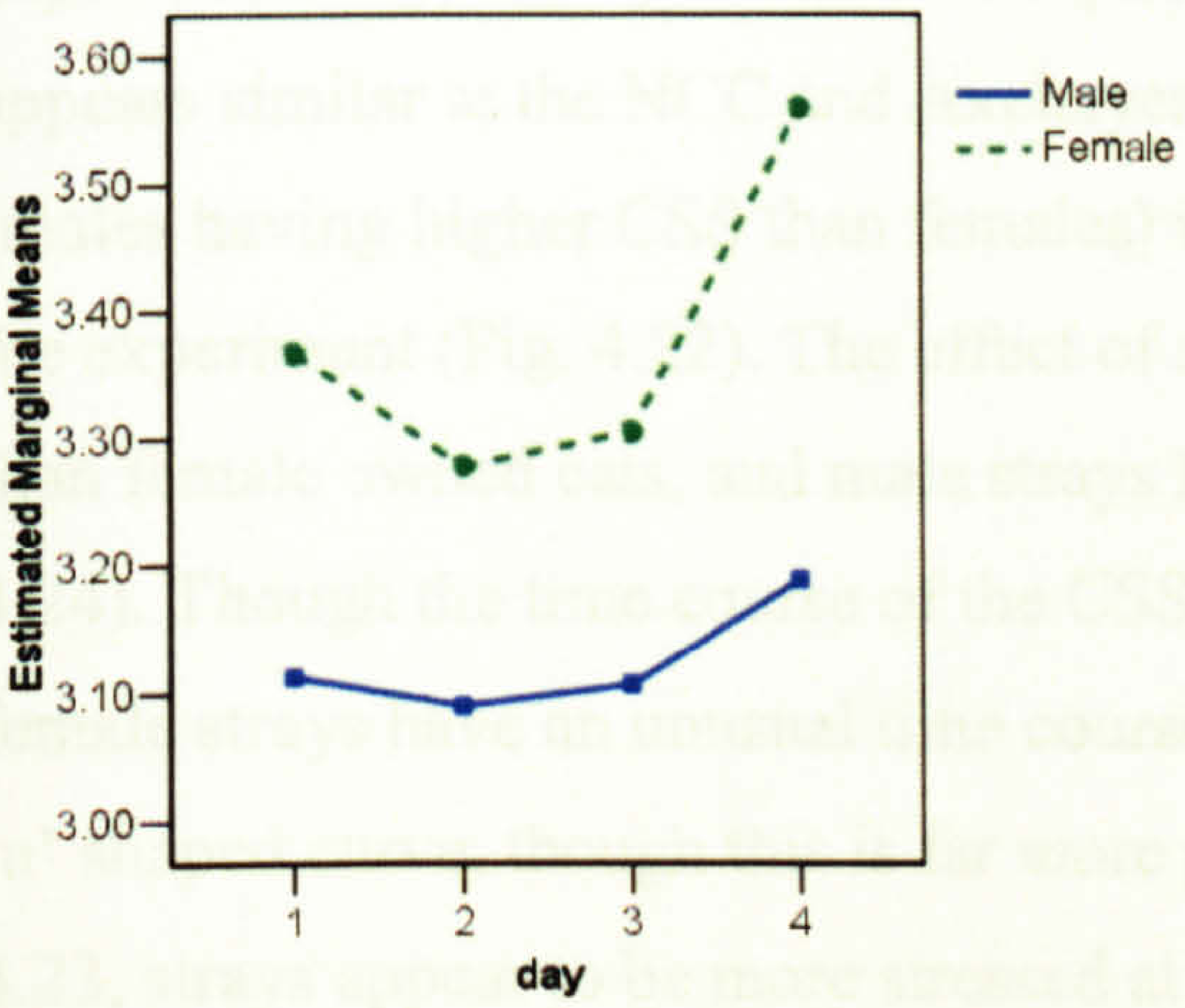


Figure 4.23 Estimated daily marginal means for CSS, split by owned vs.stray, Axehayes and NCC only

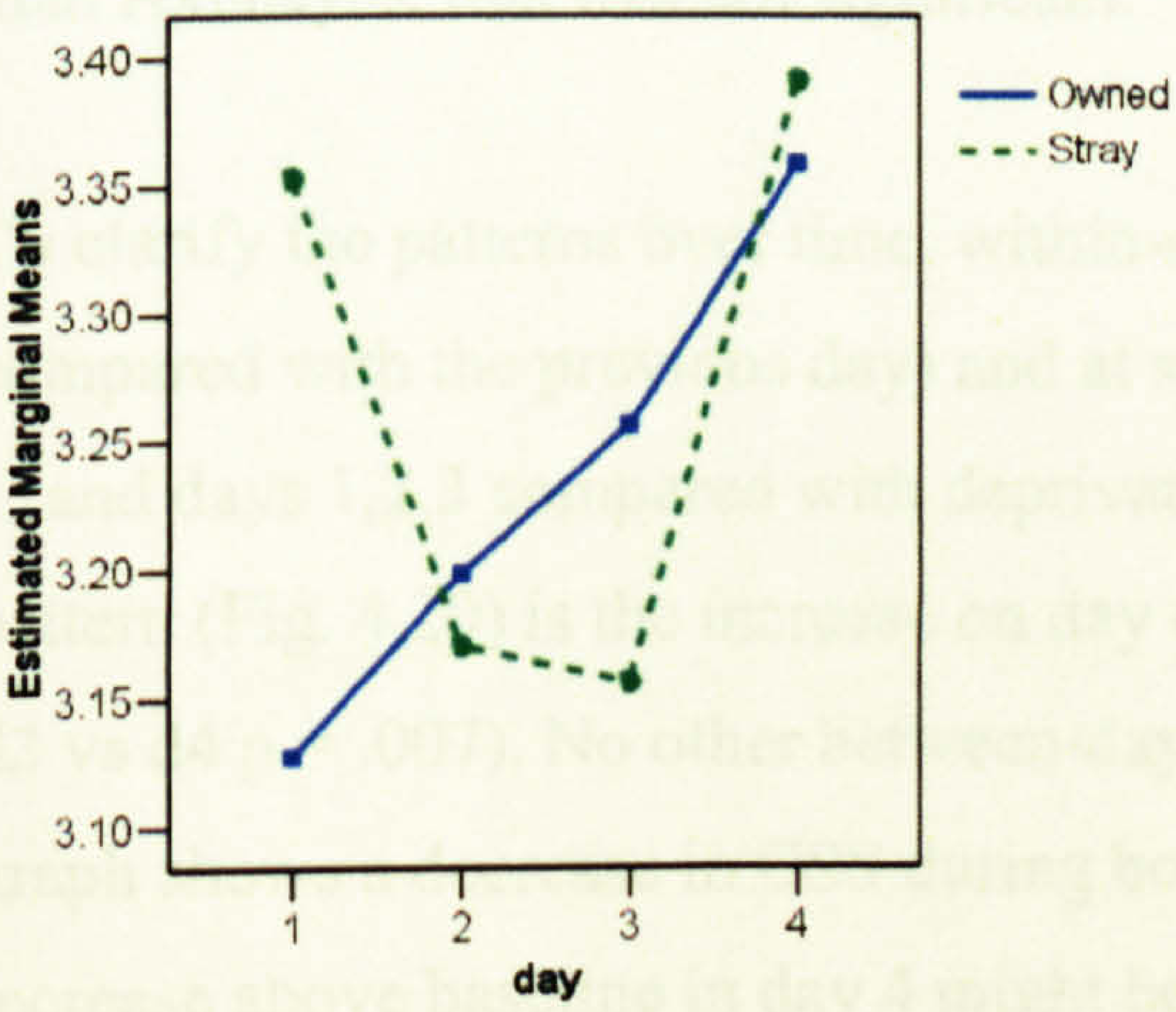
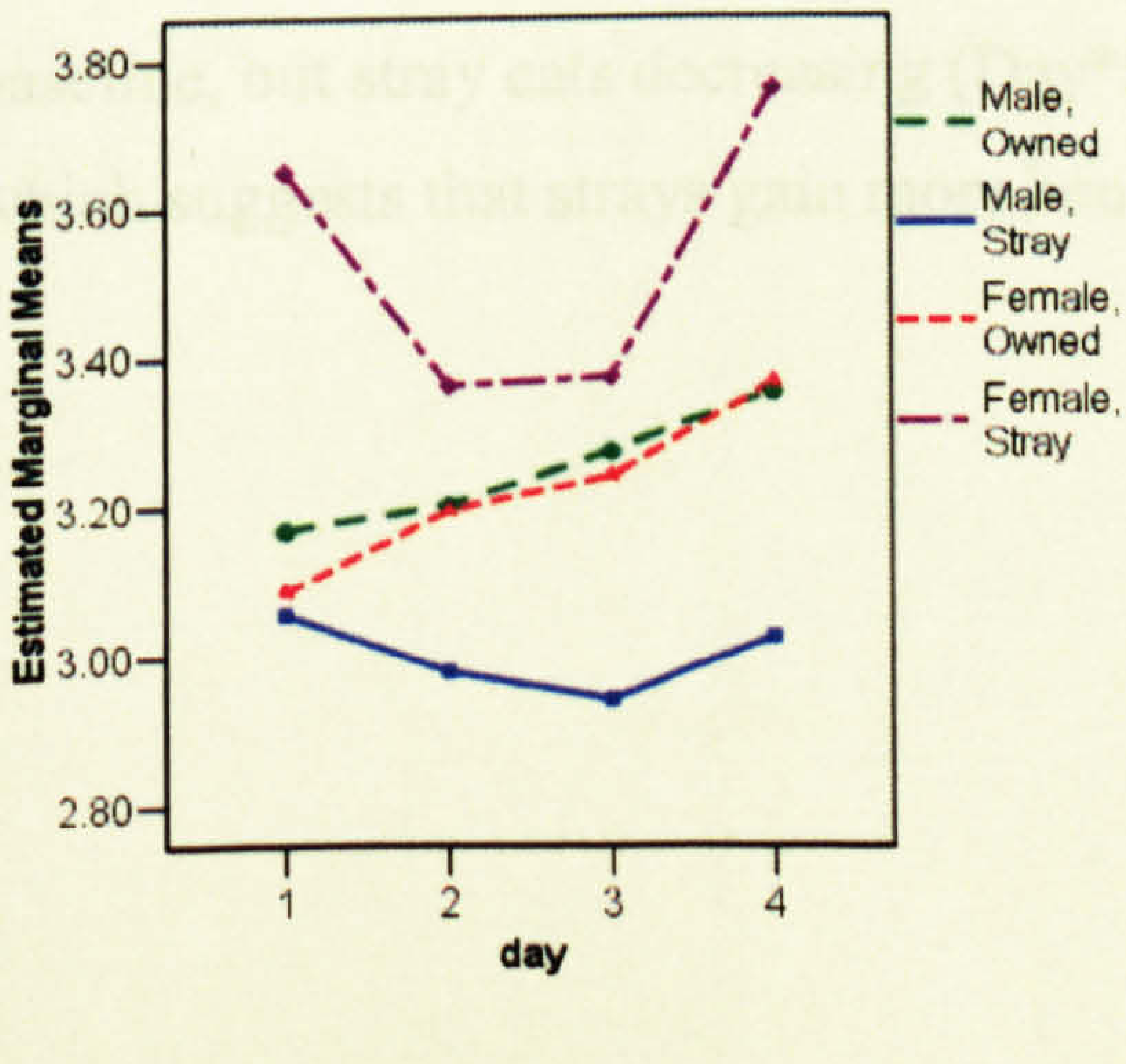


Figure 4.24 Estimated marginal means for CSS, split by sex*stray, Axehayes and NCC only



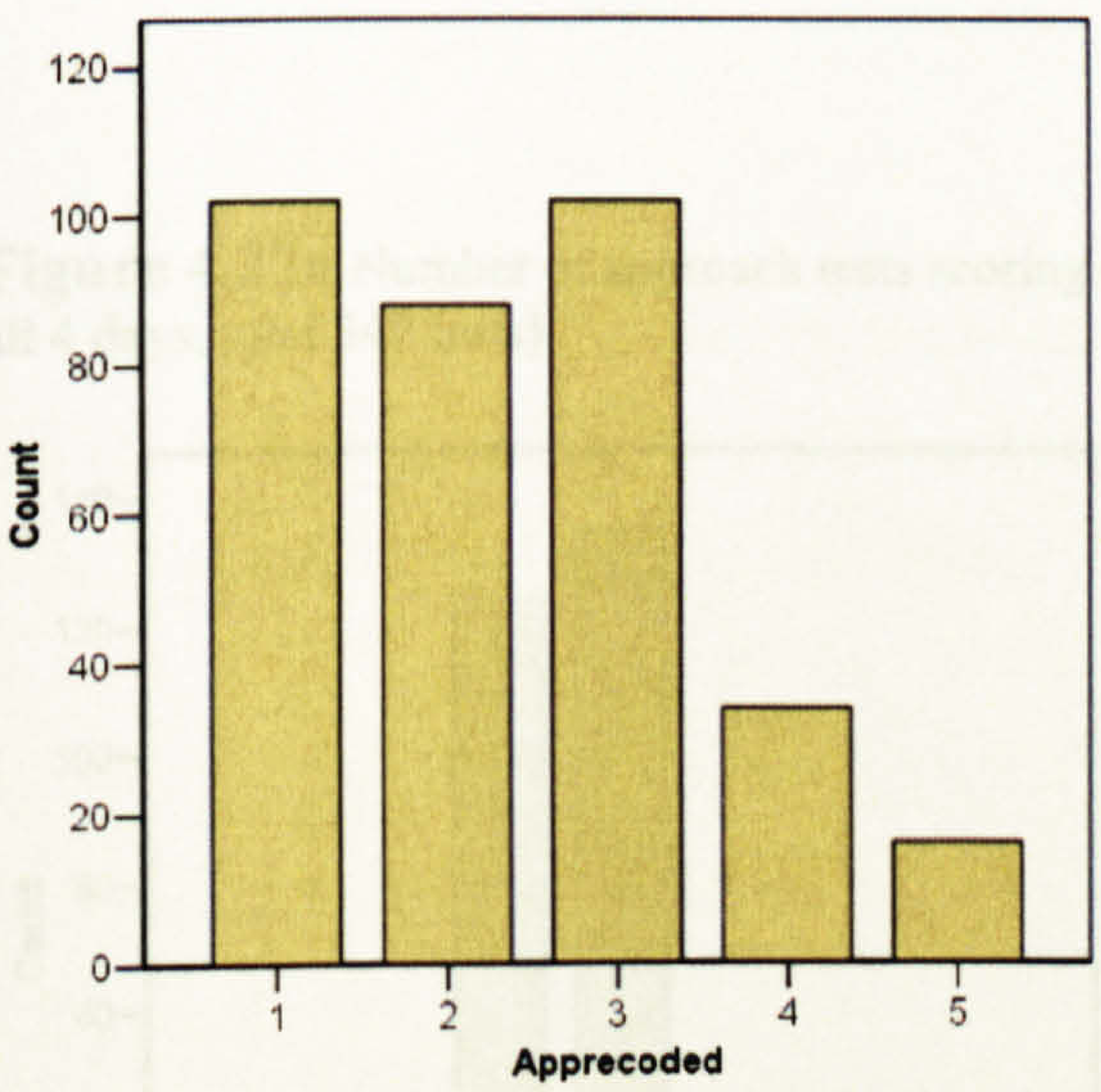
Overall (Fig. 4.20) the presence of the box on days 2 and 3 appears to be lowering the average stress-score, though even the greatest difference (between day 2 and the deprivation day, day 4) is less than a quarter of a scale-point. The effect of the box appears similar at the NCC and Axehayes shelters (Fig. 4.21). The effect of sex (males having higher CSS than females) is straightforward and seems constant across the experiment (Fig. 4.22). The effect of stray is not: female strays have higher CSS than female owned cats, and male strays have lower CSS than male owned cats (Fig. 4.24). Though the time course of the CSS of male strays is similar to owned cats, female strays have an unusual time course. For both male and female strays, there is a 'u' shaped curve, though this is far more pronounced for female strays. From Fig. 4.23, strays appear to be more stressed at baseline, but are calmed more by the box than owned cats, which would suggest that strays have more of a need to hide from an observer. This may be due to strays being generally less socialised to humans and may therefore gain more benefit from the box. Although NCC has a slightly higher CSS than Axehayes, this was not significant.

To clarify the patterns over time, within-subjects contrasts set at repeated (each day compared with the previous day) and at simple (days 2,3,4 compared to baseline, day 1, and days 1,2,3 compared with deprivation, day 4) were carried out. The main pattern (Fig. 4.20) is the increase on day 4 (Day, d1 vs d4 $p = .031$, d2 vs d4 $p = .001$, d3 vs d4 $p = .007$). No other between-day contrasts were significant, so although the graph shows a decrease in CSS during box days, this is not significant. The significant increase above baseline in day 4 might be stress caused by deprivation, or it might be stress caused by an increase of people at the weekend which had been masked by the box on day 3. There was a significant within-subjects difference between stray and owned cats on days 2 and 3 however with owned cats increasing with respect to baseline, but stray cats decreasing (Day*Stray, d1 vs d2 $p = .046$, d1 vs d3 $p = .010$) which suggests that strays gain more benefit from the box.

4.3.3 Approach test

Data from all cats and all days was pooled (342 tests) to obtain a gross overview of the variables. *Appclosed* had 102 scores of 'approach', 16 of 'withdraw' and 224 of 'neither'. *Appopen* had 190 scores of 'approach', 48 'withdraw' and 104 'neither'. More cats showed a reaction when the door was opened than when it was closed – cats in shelters presumably learn that approaches, especially by members of the public, often do not result in entrance to the cage and ignore them. A histogram of *apprecoded* is below (Figure 4.25).

Figure 4.25 Number of approach tests scoring each class of *apprecoded* (pooled data from all cats and all 4 days, total 342 tests). Scores as in section 4.2.7



All values, from 1 "very friendly" to 5 "very fearful", were recorded for *friendliness*, with "very friendly" the largest category (Fig. 4.26). Values of *CSSstart* and *CSSfin* had a similar range to those recorded for CSS during the scans (Figs. 4.27a and 4.27b respectively).

Figure 4.26 Number of approach tests scoring each class of *friendliness* (Pooled data from all cats and all 4 days, total 338 tests). Scores as in section 4.2.8.

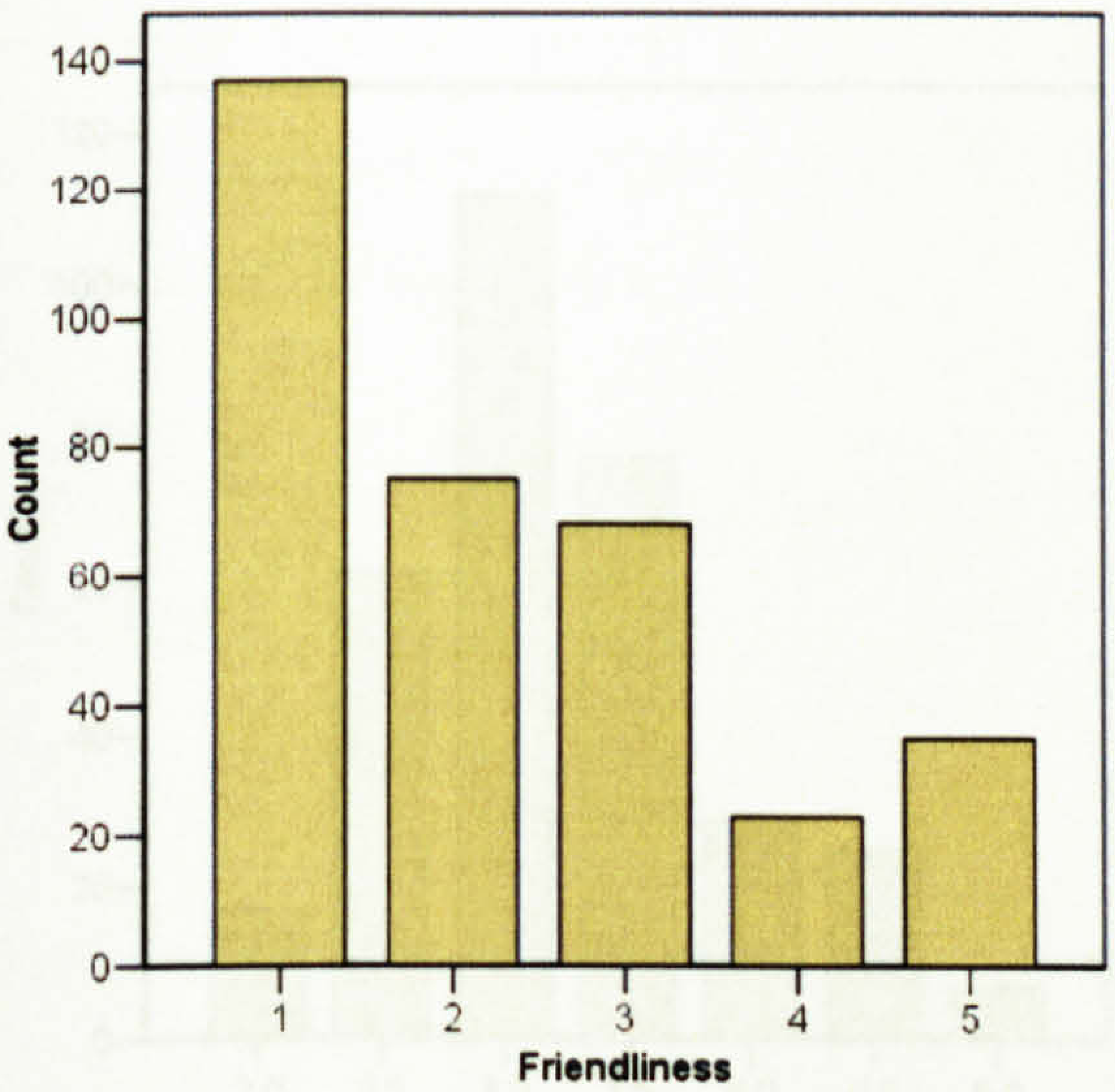


Figure 4.27a Number of approach tests scoring each class of *CSSstart* (pooled data from all cats and all 4 days, total 342 tests).

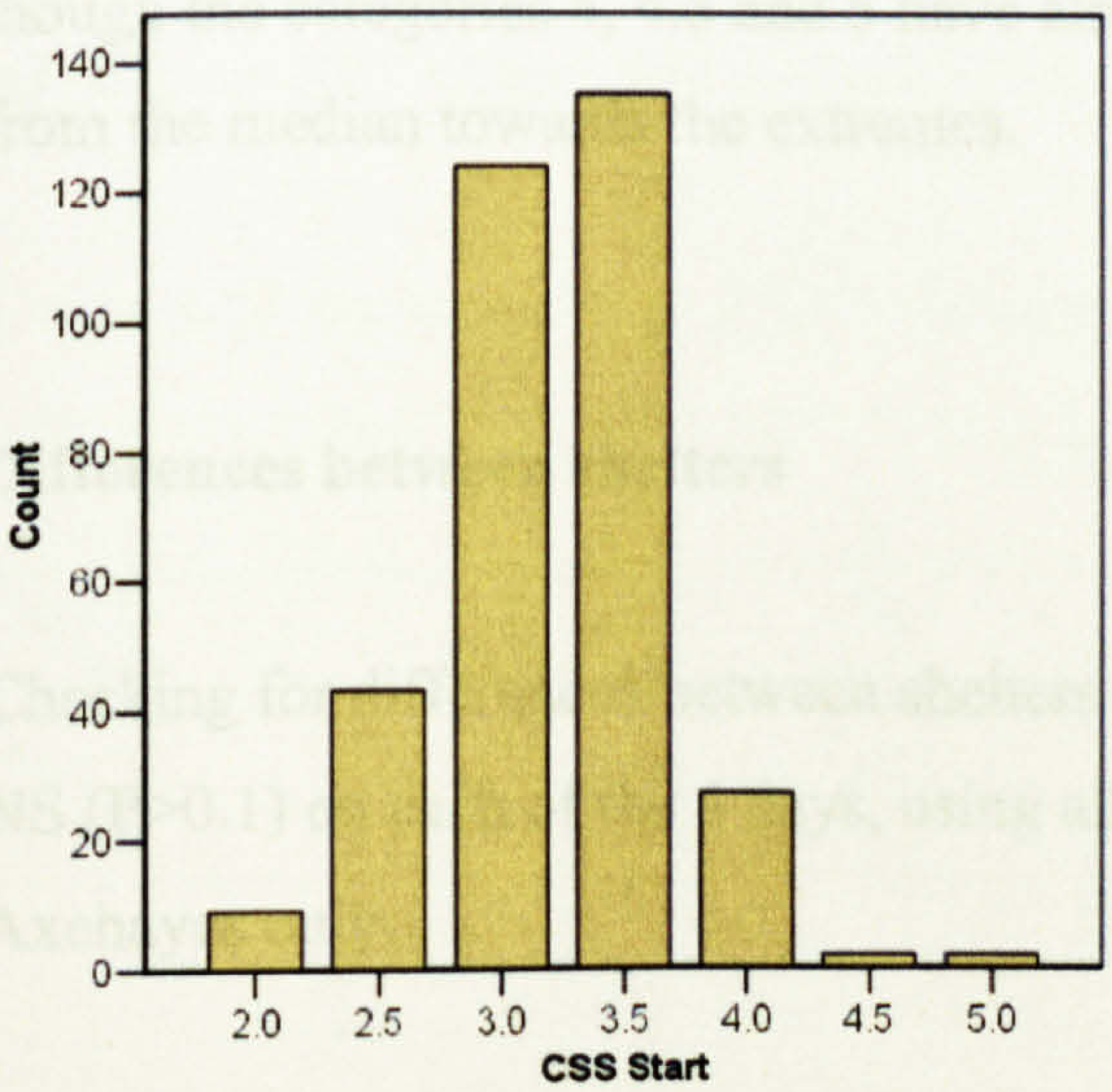
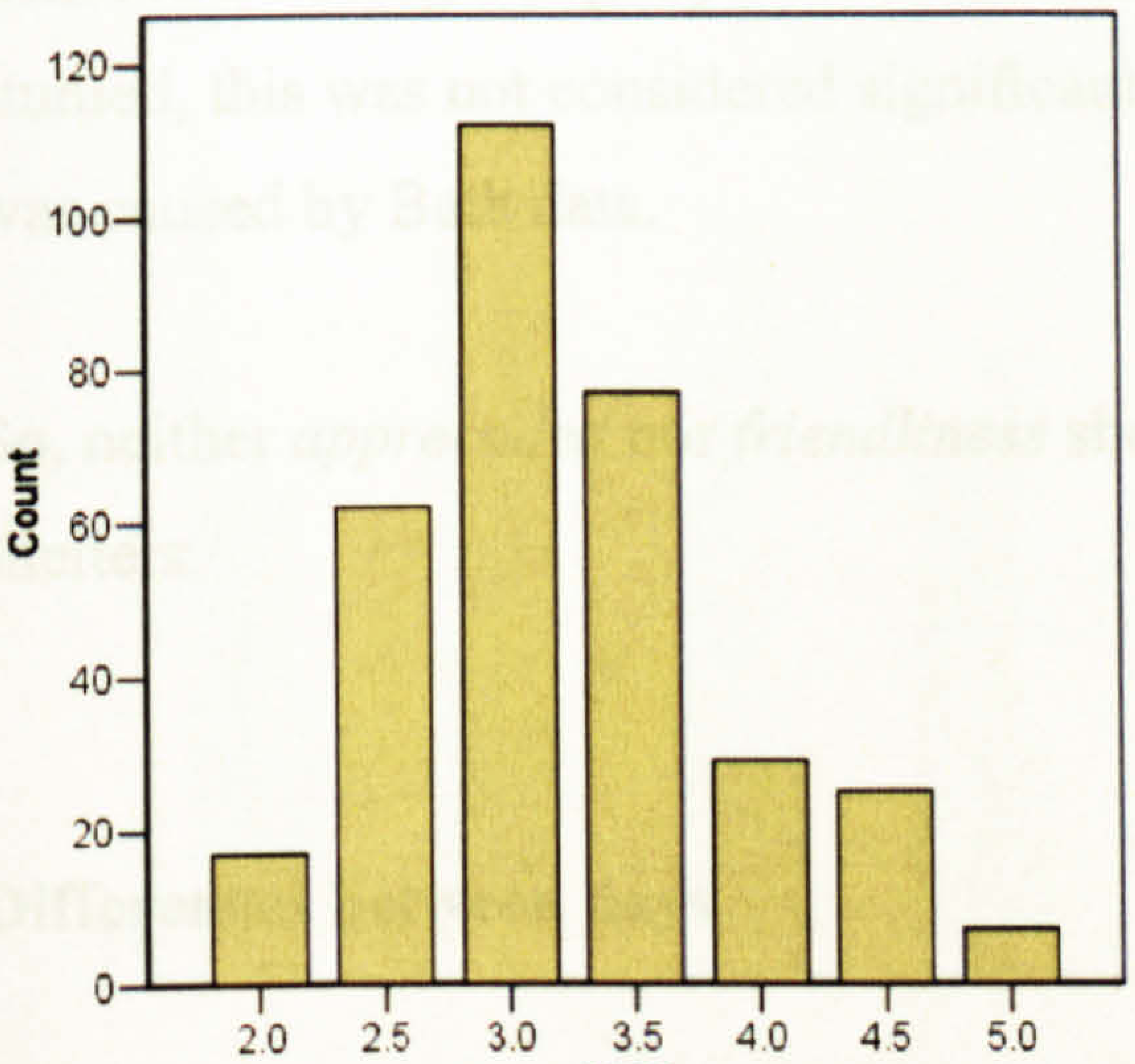


Figure 4.27b Number of approach tests scoring each class of *CSSfin* (pooled data from all cats and all 4 days, data from 329 tests - lower than *CSSstart* as cats occasionally reacted to staff, other cats, the public etc. towards the end of the test)



Comparing *CSSfin* and *CSSstart*, the peak of *CSSfin* appears to have moved to the left, though the categories 4, 4.5 and 5 have also increased. This may indicate a shift away from the median towards the extremes.

Differences between shelters

Checking for differences between shelters using chi-squared tests, *Apprecoded* was NS (P>0.1) on each of the 4 days, using all 3 shelters' data, and also for NCC and Axehayes only.

Checking for differences using K-W tests for *friendliness* and the two CSS variables, days 1 and 2 were NS for all variables, though days 3 and 4 were significant for both *CSSstart* and *CSSfin* (day 3 *CSSstart* p=.017, *CSSfin* p=.009; day 4 *CSSstart* p=.002, *CSSfin* p=.000). In all cases, Bath had lower CSS. Bath *CSSstart* and *CSSfin* data were removed from the analysis (q.v. Section 4.3.2, removal changes the significance of none of the results), though Bath data was retained for *CSSupdown*.

Using Mann-Whitney tests to look at differences between NCC and Axehayes, days 1, 2 and 4 were NS for all variables at $p > 0.1$, day 3 had $p = .014$ for *CSSstart* ($p=.014$), with NCC having a slightly lower CSS. Given that 4 days and three variables were studied, this was not considered significant, and confirms that the difference above was caused by Bath data.

So, neither *apprecoded* nor *friendliness* showed a significant difference between shelters.

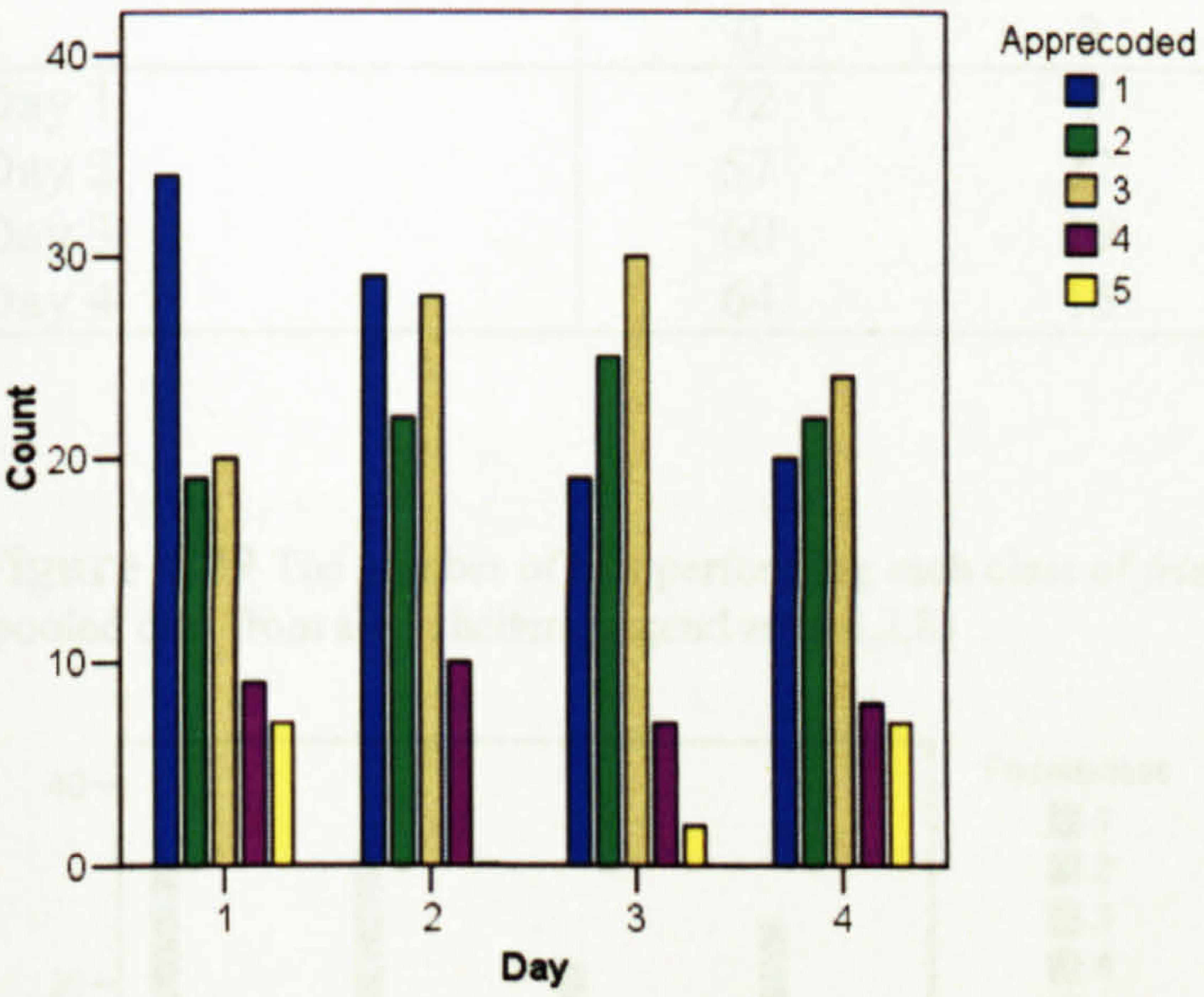
Differences between days

Apprecoded The Friedman's test was NS at $p = .227$. All pairs of days were $p > 0.1$ apart from d1 and d3, $p = .045$. After correcting for multiplicity on 6 day pairs, this was also NS. Looking at individual classes (Fig. 4.28), all classes were NS at $p > 0.1$ apart from score '1', $p = .083$, and score '5', $p = .005$. Score 5 was the only score significant after accounting for multiplicity, decreasing during box days (Table 4.14).

Table 4.14 *Apprecoded* scoring '5' (withdrawal during the 1st phase) or '0' (not 5)

	Value	
	0	5
Day 1	74	7
Day 2	81	0
Day 3	79	2
Day 4	74	7

Figure 4.28 The number of cats scoring each class of *apprecoded* on each of the four days (pooled data from all 3 shelters, legend as in section 4.2.8)



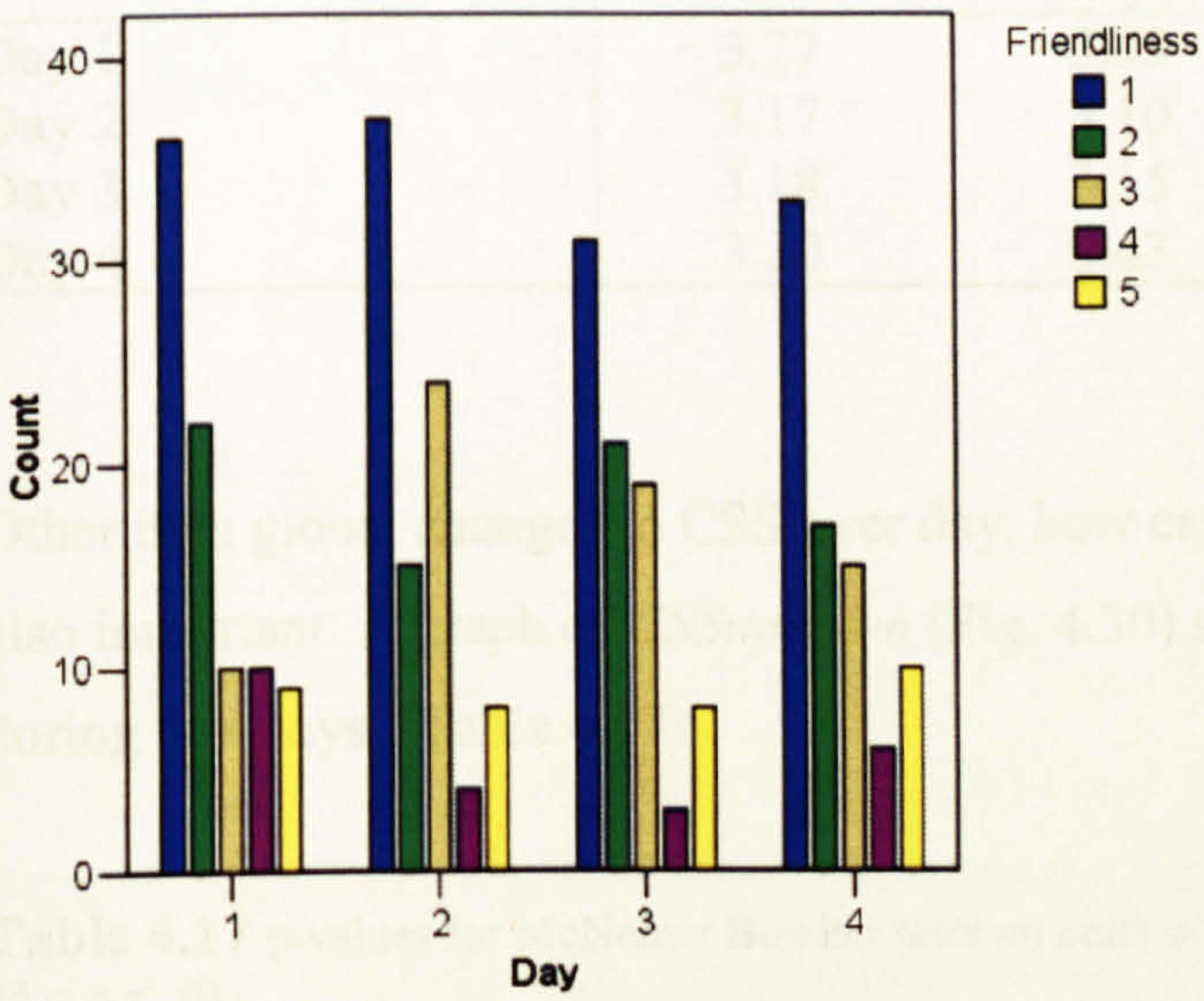
Although *apprecoded* did not change over time overall, there was a significant effect on score '5' (Withdrawal in the first phase) which is lower on box days, as shown in the graph. It appears to have been replaced by an increase in score '3' (ignore) which is higher on box days, though this was not significant. The reduction in '5' was not replaced by an increase in '4', though it is possible that the boxes moved some cats from '5' to '4', and some cats from '4' to '3', the change being harder to detect amongst the higher N of scores '3' and '4'. All cats that scored 5 left the pod and went out into the run, so this shows that boxes significantly reduce the number of cats fleeing, which may be useful for rehoming them, and may make some caretaking activities such as routine check ups and taking the cat to a veterinarian more simple. Score '1' appears to decrease over the course of the experiment, though the p-value for this was only $p = .083$. Though this suggests that the cats are less willing to approach in the first phase over time (possibly the cats learn that the author is a poor companion), it is not proven.

Friendliness The Friedman's test was NS at $p = .964$. All pairs of days were NS at $p > 0.1$. All classes were $p > 0.1$ apart from score '3' $p = .009$ and score '4' $p = .089$. Score 3 was the only score significant after accounting for multiplicity (Table 4.15), increasing during box days (Fig. 4.29). *Apprecoded* data (Fig. 4.28) follows the same trend, but is NS.

Table 4.15 *Friendliness* scoring '3' (neither friendly nor unfriendly) or '0' (not 3)

	Value	
	0	3
Day 1	72	7
Day 2	57	22
Day 3	60	19
Day 4	64	15

Figure 4.29 The number of cats performing each class of *friendliness* on each of the four days (pooled data from all 3 shelters, legend as in 4.2.8)



Friendliness score '3' significantly increases during box days, and appears to accompany a decrease in '4', so boxes make cats less likely to approach or withdraw. The increase is not just days 2 and 3, it is also slightly higher than baseline on day 4. This suggests that the effect might partly be the cats learning that the observer was unresponsive and not approaching him for that reason. Score '4' appears to decrease during box days, though this was NS.

CSS The Friedman test on *CSSfin* was sig at p = .000, *CSSstart* was NS at p=.207, though shows a similar trend (Table 4.16): *CSSfin* is lower during box days, as is *CSSstart* (though not significantly). With comparison to the histogram of *CSSfin* (Fig. 4.27b) it looks as if the entire curve is shifted to the to the left a little on box days. So *CSSfin* is significantly lower on box days. This shows that the box makes cats less stressed by the approach test.

Table 4.16a Friedman tests on *CSSstart* and *CSSfin*. See text for p-values.

	Mean rank	
	CSSstart	*CSSfin*
Day 1	2.55	2.69
Day 2	2.36	2.11
Day 3	2.40	2.25
Day 4	2.69	2.95

Table 4.16b Mean values for *CSSstart* and *CSSfin*.

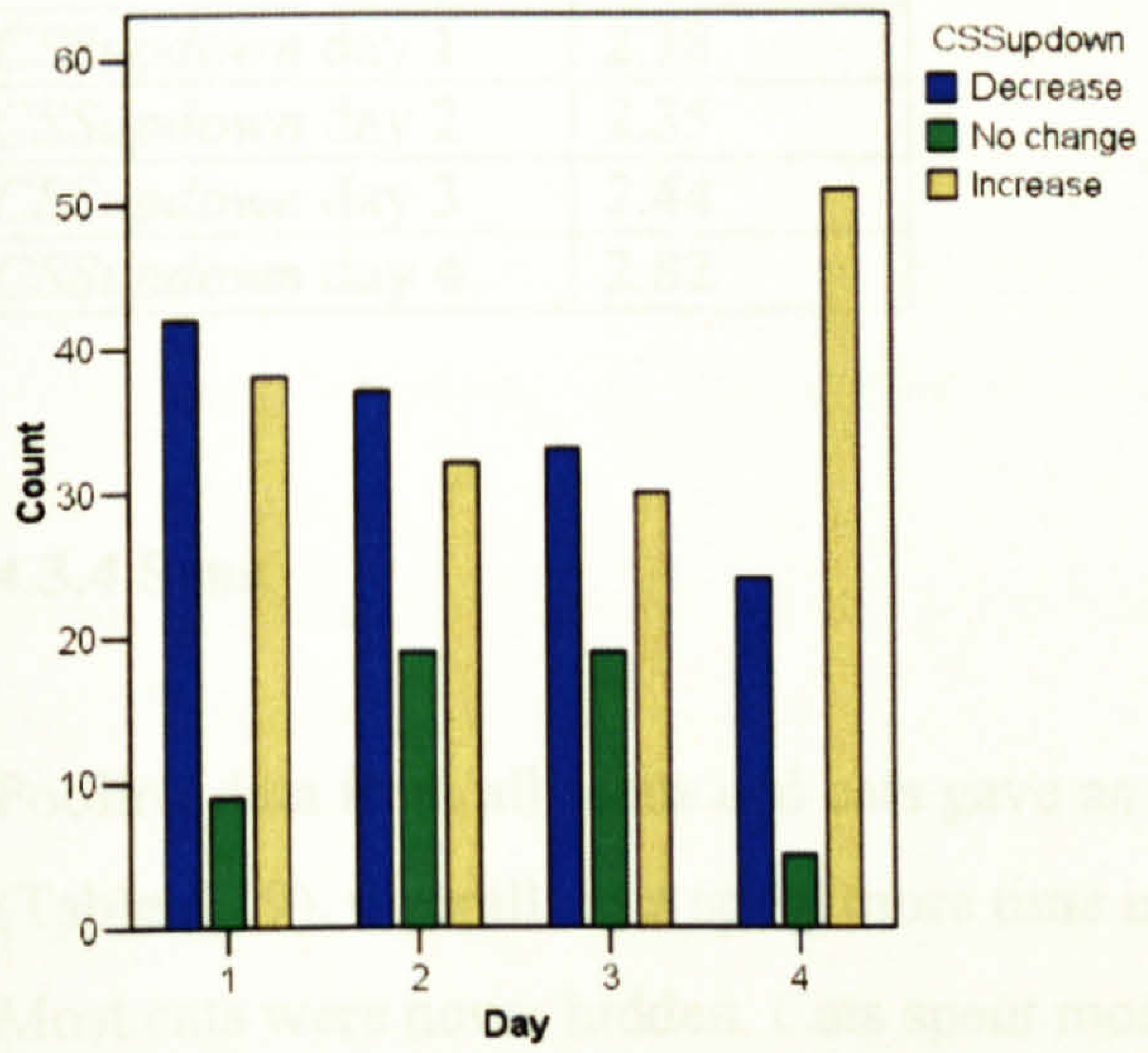
	Mean value	
	CSSstart	*CSSfin*
Day 1	3.27	3.33
Day 2	3.17	3.10
Day 3	3.18	3.15
Day 4	3.30	3.43

Other than global changes to CSS over day, how cats respond to the approach test is also important. A graph of *CSSupdown* (Fig. 4.30) seems to indicate more 'no change' during box days. (Table 4.17).

Table 4.17 p-values for McNemar Bowker tests on pairs of days of *CSSupdown*. * = $p < .05$, ** = $p < .01$

Days tested	p-value
dl v d2	.142
dl v d3	.040
dl v d4	.023
d2 v d3	.627
d2 v d4	.001
d3 v d4	.001

Figure 4.30 The number of cats performing each class of *CSSupdown* on each of the four days (pooled data from all 3 shelters)



In all of the significant cases with day 2 or 3, the box days had higher levels of no change, and lower levels of decrease and increase. The difference between day 1 and 4 is that day 4 had less cats with a decrease over the test, and more with an increase, so cats become more stressed during the approach test on day 4 than on day 1. This suggests that boxes reduce the effect of the approaching observer on CSS regardless of the cat's opinion (positive or negative) of the observer. The high level of 'increase' on the deprivation day suggests that cats respond more negatively to the approach test when the box has been removed.

Treating *CSSupdown* as ordinal (scoring a decrease compared to the previous day as '0', increase as '1' and no change '0.5') and then conducting a Friedman test gave $p=.007$. The main difference appears to be between day 4 and all other days, with more cats on day 4 scoring an increase, and less a decrease (Table 4.18). Wilcoxon signed ranks tests on pairs of days are NS at $P > 0.1$ apart from comparisons with day 4; all comparisons with day 4 have $p <.01$, with day 4 being higher. This agrees with the McNemar-Bowker tests above (Table 4.17).

Table 4.18 Mean ranks of Friedman test for *CSSupdown* scored as ordinal data, for days 1-4.

	Mean rank
CSSupdown day 1	2.38
CSSupdown day 2	2.35
CSSupdown day 3	2.44
CSSupdown day 4	2.82

4.3.4 Scan

Pooling data from all scans and cats gave an overview of how cats spent their time (Table 4.19). Overall, cats spent more time inside the pods than outside in the runs. Most cats were never hidden. Cats spent most of their time facing the front, and had their heads towards the front of the pod. Cats spent most of their time resting with their eyes open, and only slightly less time resting with their eyes shut. Most were fully exposed, and spent most of their time lying ventrally, with tense sleep being the second most common posture. Most cats when inside spent their time towards the back of the cage, and cats which were outside were generally either hiding under the ramp or sitting fully exposed.

Table 4.19 Results from scan tests (proportions), pooled data from all cats and days. Legend as in section 4.2.7.

Class	InOut	Hide	HfBf	Face	Behav	Exposed	Posture
'1'	.88	.96	.71	.59	.02	.55	.02
'2'	.12	.04	.29	.14	.54	.13	.13
'3'				.22	.41	.28	.43
'4'				.06	.03	.03	.34
'5'					.01	.02	.03
'6'							.02

	Position, InOut = In	Position, InOut = Out
'1'	.26	.23
'2'	.08	.03
'3'	.59	.63
'4'	.05	.04
'5'	.02	.07

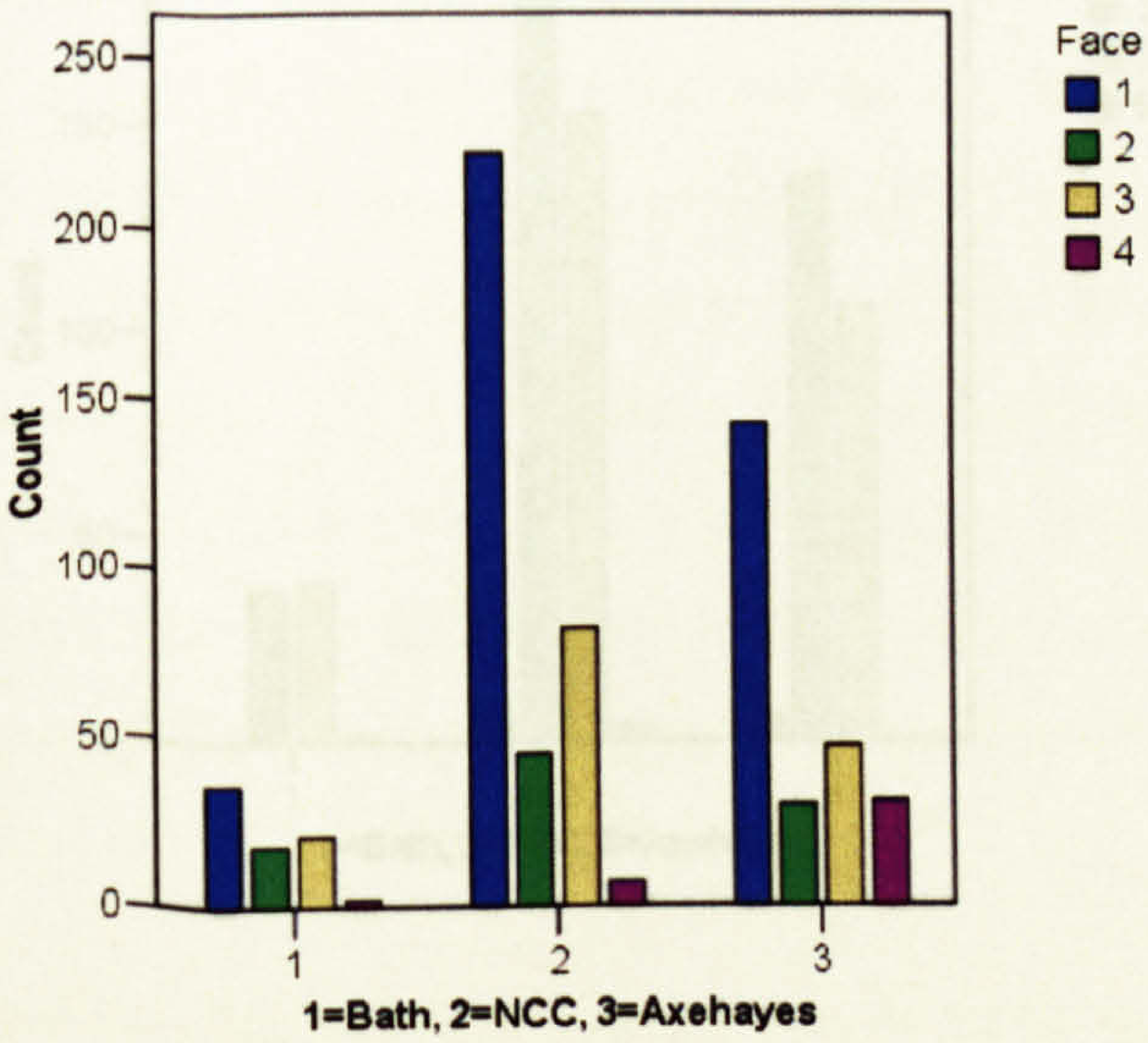
Differences between shelters

InOut: Testing all 3 shelters, day 3 morning (m3) had p=.016 (significance seemed to be due to NCC having a large proportion of cats out in the run). The rest were all NS at p>0.1. Including multiplicity of 8 scans, the m3 result is not significant. The same was found with just NCC and Axehayes, with m3 having p = .018.

Hide: Testing all 3 shelters, all scans were NS (p>0.1). The same was found for NCC and Axehayes only.

Face: For all 3 shelters, morning 1 (m1) p = .007, day 1 afternoon (a1) p = .024, a2 p = .032 and m4 p = .000. The rest were all NS, p > 0.1. For NCC and Axehayes, m1 p = .010, a1 p = .019, m2 p = .094, a2 p = .011, m4 p = .008 and a4 p = .082, the rest were NS at p > 0.1. By inspection, Axehayes often had higher proportion of '4', presumably due to the window at the back of the pods (Fig. 4.31).

Figure 4.31 The number of scan tests in which each class of *face* was performed, split by shelter (pooled data from all tests over the 4 days, legend in section 4.2.7)

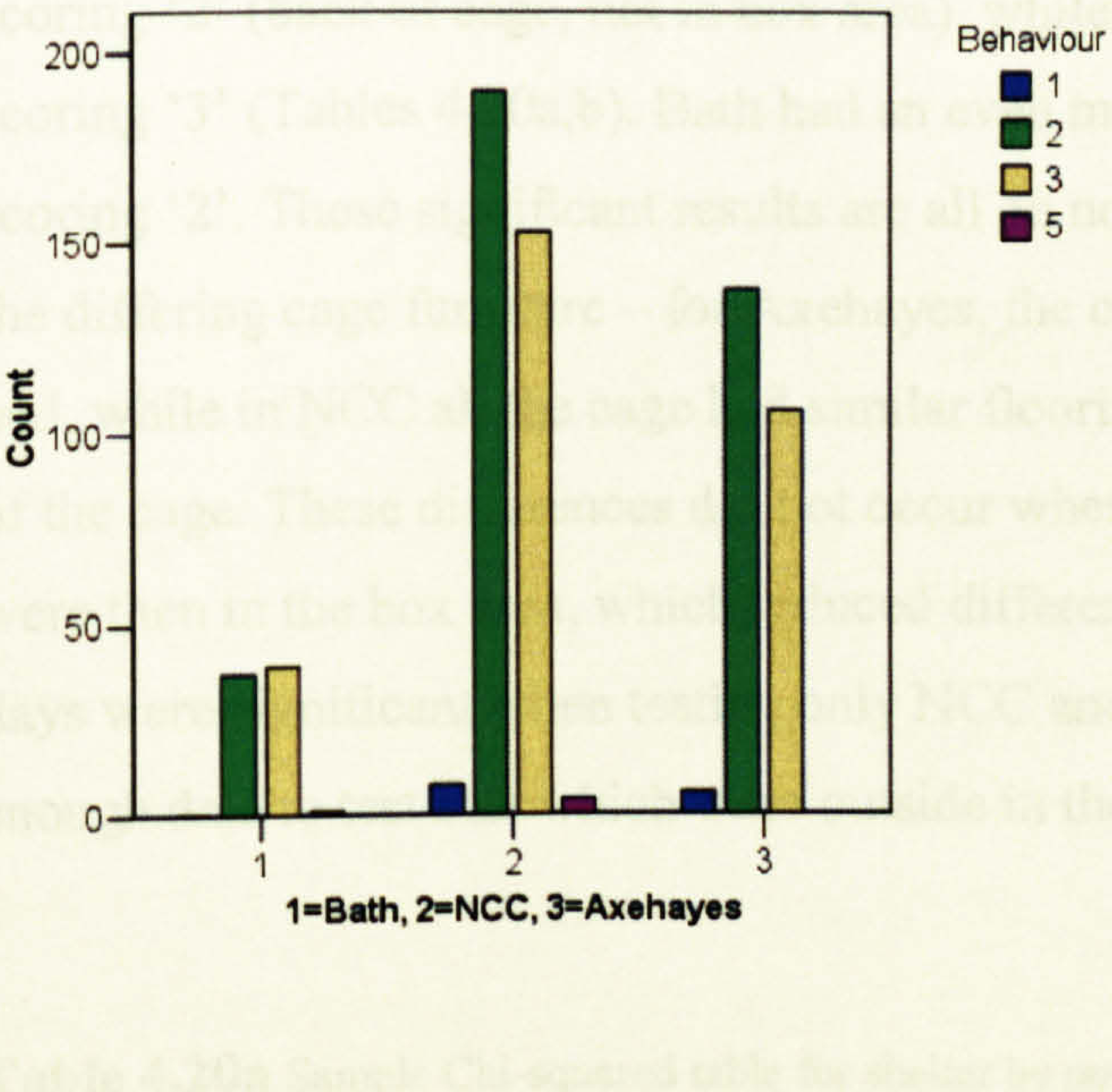


Class '4' was recoded as missing data and the analysis repeated. This still left a1, a2 and m2 significant, this time with NCC scoring highly as '1' or '3'. Since it is harder for Axehayes cats to score a 3 than for cats at the other shelters, and taking into account the effect of the window, it is likely that *face* would remain different between shelters even with further recoding of the data.

Behaviour: Testing all 3 shelters, m1 $p = .052$, and m4 $p = .008$. All other morning scans and all afternoon scans were NS, $p > 0.1$. Looking at the apparent differences showed that in m1, Bath scored highly in '3' and low in '2', and in m4 Bath scored highly in '2' and low in '3' compared to NCC and Axehayes. This variation, coupled with m1 being over the usual $p = .05$ boundary, suggests that there is not a systematic difference. This is backed up by Fig. 4.32, which shows no obvious differences between shelters.

Looking at data from NCC and Axehayes only, m2 $p = .096$ and m3 $p = .069$, the rest were NS at $p > 0.1$. This confirms the visual inspection of the data that the low p-values on days 1 and 4 were due to Bath cats. It may be that there was something environmental that happened during the morning changed their behaviour but it could equally well be chance.

Figure 4.32 The number of scan tests in which each class of *behaviour* was performed, split by shelters (pooled data from all tests over the 4 days, legend in section 4.2.7)



Exposed: Very similar results were found for all three shelters and for NCC and Axehayes: m1, a1, m4 and a4 were all significant at $p < .0005$, the rest being NS at $p>0.1$. This is probably due to Axehayes having a bed (which was scored a 2 unless the cat was sitting up) and the others a blanket (scored as a 1). Recoding 'Exposed' such that bed = 1 and repeating the analysis, m1 had $p = .093$ and $p = .052$ for all three and NCC and Axehayes respectively (Axehayes had slightly more cats scoring

‘3’, possibly because the beds made it easier for cats’ heads to be hidden), the rest were all NS at $p > 0.1$, so there was no significant difference overall.

***Hfbf*:** This measure was recorded for NCC and Axehayes only. All scans were NS at $p > 0.1$ except for: m1 $p = .047$; m3 $p = .011$ and a4 $p = .031$. In all three of these cases, NCC had a higher proportion facing forward, Axehayes a higher proportion facing back. This may be due to the back wall in Axehayes being a window, so cats may have been looking back due to the view rather than avoiding looking at the observer.

***Posture*:** This measure was recorded for NCC and Axehayes only. All days were NS at $p>0.1$.

***Position*:** Looking at ‘inside’ cats only, all scans had $p > 0.1$, except for m1 $p = .001$, a1 $p = .001$, m4 $p = .007$ and a4 $p < .001$. In all cases, NCC had a large number of cats scoring ‘1’ (front of cage), some scoring ‘3’ (back of cage, in box/bed area), and a few scoring ‘2’ (back of cage, not in box area), while Axehayes had a large number of cats scoring ‘3’ (Tables 4.20a,b). Bath had an even mix of ‘1’ and ‘3’, with a few cats scoring ‘2’. These significant results are all on non-box days, and probably stem from the differing cage furniture – for Axehayes, the cats were used to resting in the cat bed, while in NCC all the cage had similar flooring, and many cats came to the front of the cage. These differences did not occur when the box was present – most cats were then in the box area, which reduced differences between the shelters. The same days were significant when testing only NCC and Axehayes data. There was not enough data to test cats which were outside in the runs.

Table 4.20a Sample Chi-squared table for shelter by *position*, m1 (typical non-box day) data, ‘in’ cats only

Position score	Bath	NCC	Axehayes
‘1’ (Front of cage)	5	17	3
‘2’ (Back of cage, not in box/bed area)	0	8	0
‘3’ (Back of cage, box/bed area)	6	11	25
‘5’ (Other)	0	2	0

Table 4.20b Sample Chi-squared table for shelter by *position*, m2 (typical box day) data, 'in' cats only

Position score	Bath	NCC	Axehayes
'1' (Front of cage)	0	5	7
'2' (Back of cage, not in box/bed area)	1	5	5
'3' (Back of cage, box/bed area)	10	28	18
'4' (On box)	0	1	0
'5' (Other)	0	1	0

Differences between days

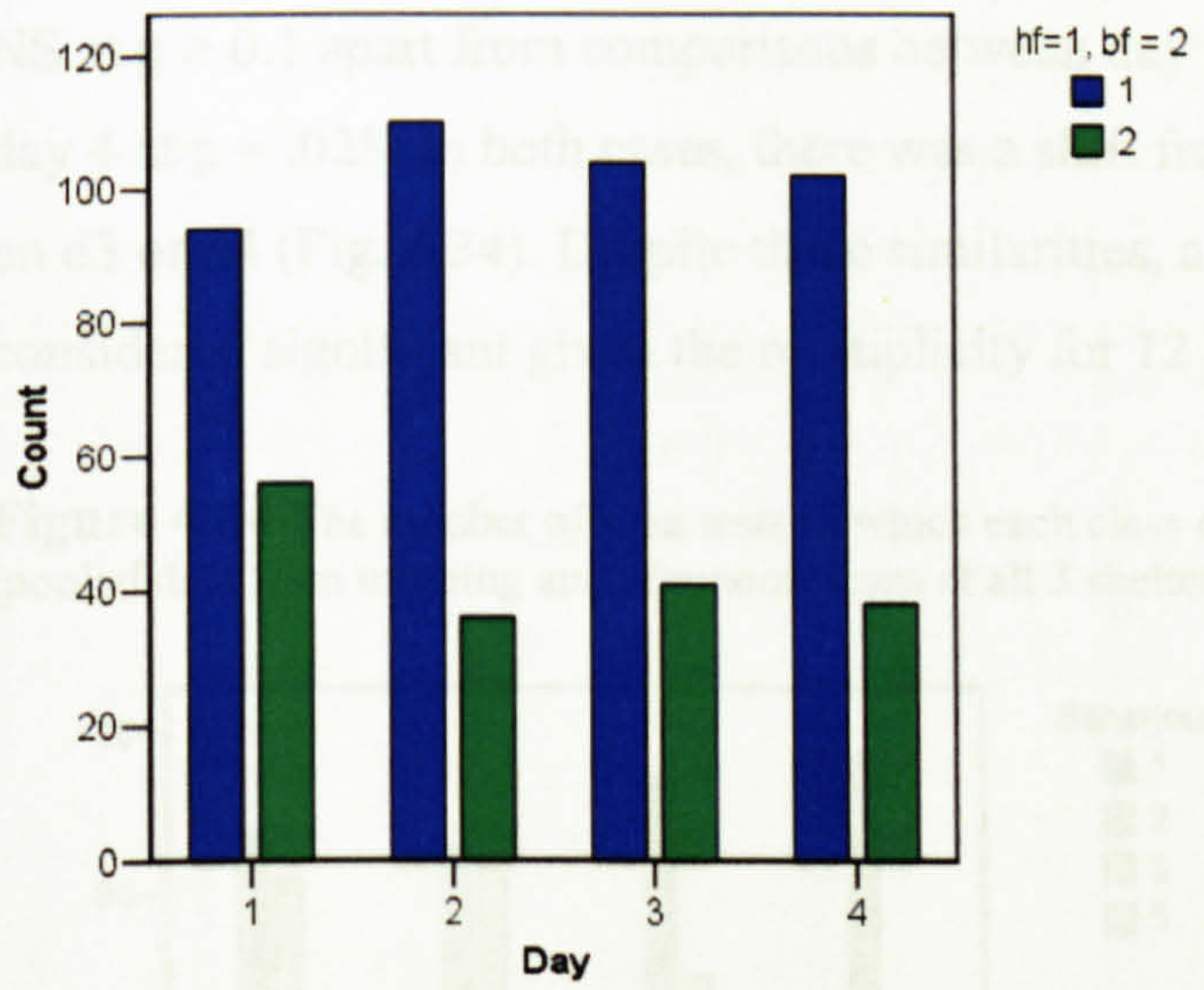
InOut Cochran's Q over the 4 days was NS at $p > 0.1$ for both afternoon and morning scans. The median of both scans per day was computed and tested with a Friedman test, and was NS at $p > 0.1$. Means were also used for testing pairs of days with Marginal Homogeneity; all were NS at $p > 0.1$.

Hide Cochran's Q over the 4 days was NS at $p > 0.1$ for both afternoon and morning scans. The mean of both scans was computed and tested with a Friedman test, which was also NS at $p > 0.1$. Means were also used for testing pairs of days with Marginal Homogeneity; all were NS at $p > 0.1$.

There is no significant difference between days for *hide* or *inout*, so the box did not affect these variables.

Hfbf Cochran's Q over the 4 days was NS at $p > 0.1$ for both afternoon and morning scans. The mean of both scans was computed and tested with a Friedman test, which was NS at $p = .053$. Means were used for comparing pairs of days with marginal homogeneity tests: d1 v d2 $p = .038$; d1 v d3 $p = .064$; d1 v d4 $p = .061$; all other day pairs were NS at $p > 0.1$. Fig. 4.33 shows that the relative proportion of head in front was lower on day 1 than the other days. The mean on day 1 was 1.36, declining to 1.25, 1.28 and 1.27 on days 2, 3 and 4 respectively.

Figure 4.33 The number of scan tests in which each class of *hfbf* was performed, split by day (pooled data from morning and afternoon scans at all 3 shelters, legend in section 4.2.7)



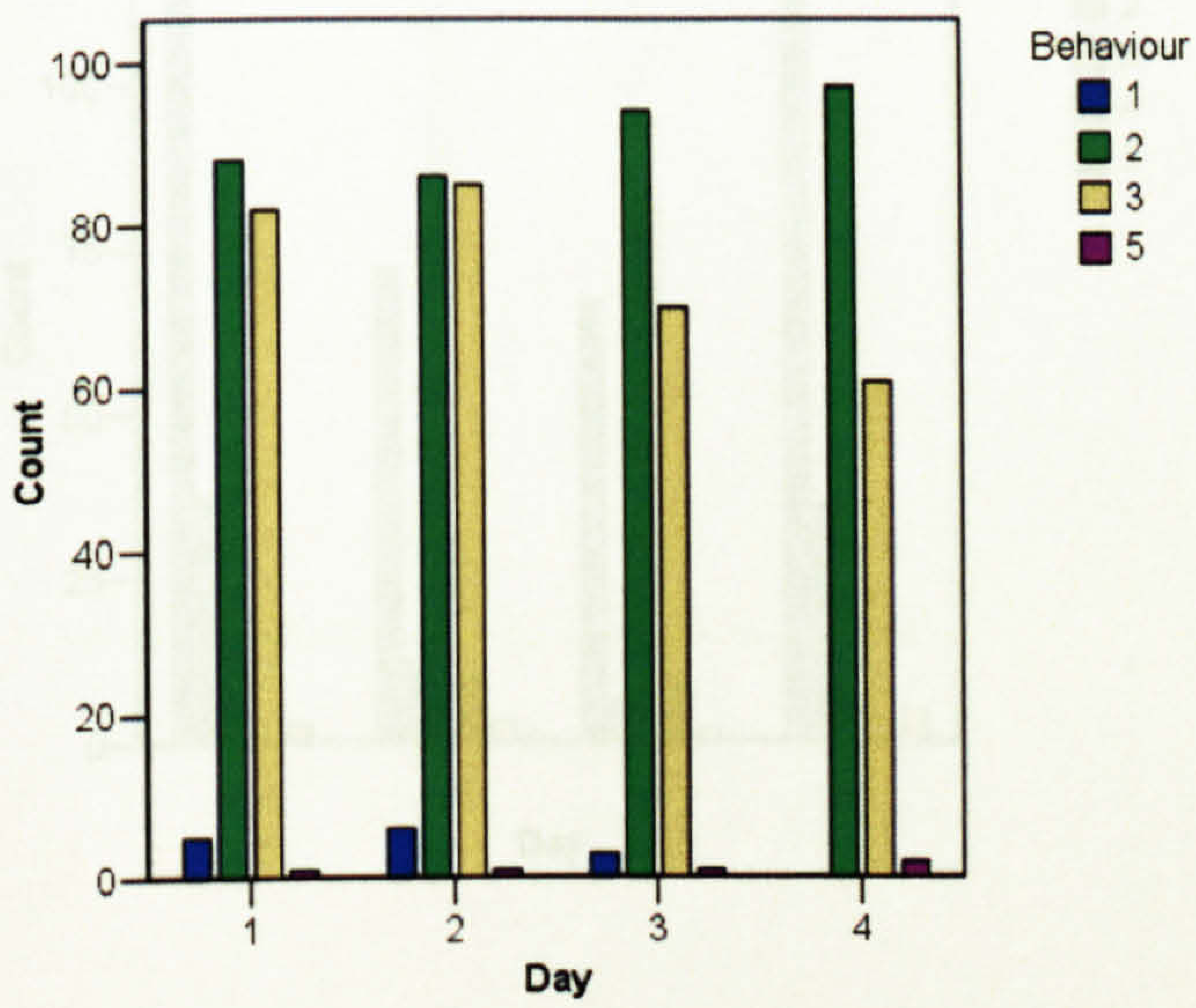
Cats on day 1 were more likely to have the body in front than on day 2; comparisons between all other pairs of days were not significant. The near-significance of comparisons between d1 and d3, and d1 and d4 suggests that the day 1 result was a response to an unfamiliar observer, and might indicate a degree of defensiveness. Since cats that are particularly interested in activities outside their cage might be expected to keep their head towards the front, this suggests that cats on day 1 were either uncertain, uninterested or hesitant, about contact with the observer.

Face After recoding so that facing front = 1, and all other facings = 0, Cochran's Q over the 4 days had $p = .081$ for morning scans, and was NS at $p > 0.1$ for afternoons. Taking the means of both scans and testing with a Friedman test gave $p > 0.1$. Means were also used for comparing pairs of days with marginal homogeneity tests: all were NS at $p > 0.1$ apart from d2 v d3 which had $p = .091$. So, there was no detected effect of either the box or deprivation on facing towards the front.

Behaviour Behaviours 1-3 are ordinal on a scale of 'alertness'. Friedman tests on all 4 days were NS at $p > 0.1$ for morning scans only, afternoon scans only, and the median of both scans for each day. The median of both scans on each pair of days was tested with marginal homogeneity tests and all were NS at $P > 0.1$.

Treating behaviour as categorical data, McNemar tests were computed for pairs of days. All pairs of morning scans were NS at $p > 0.1$. All pairs of afternoon scans were NS at $p > 0.1$ apart from comparisons between day 2 and day 3 $p = .067$, day 2 and day 4 at $p = .028$. In both cases, there was a shift from score '3' on day 2 to score '2' on d3 or d4 (Fig. 4.34). Despite these similarities, a p-value of $p = .028$ cannot be considered significant given the multiplicity for 12 pairs of scans tested.

Figure 4.34 The number of scan tests in which each class of *behaviour* was performed, split by day (pooled data from morning and afternoon scans at all 3 shelters, legend in section 4.2.7)

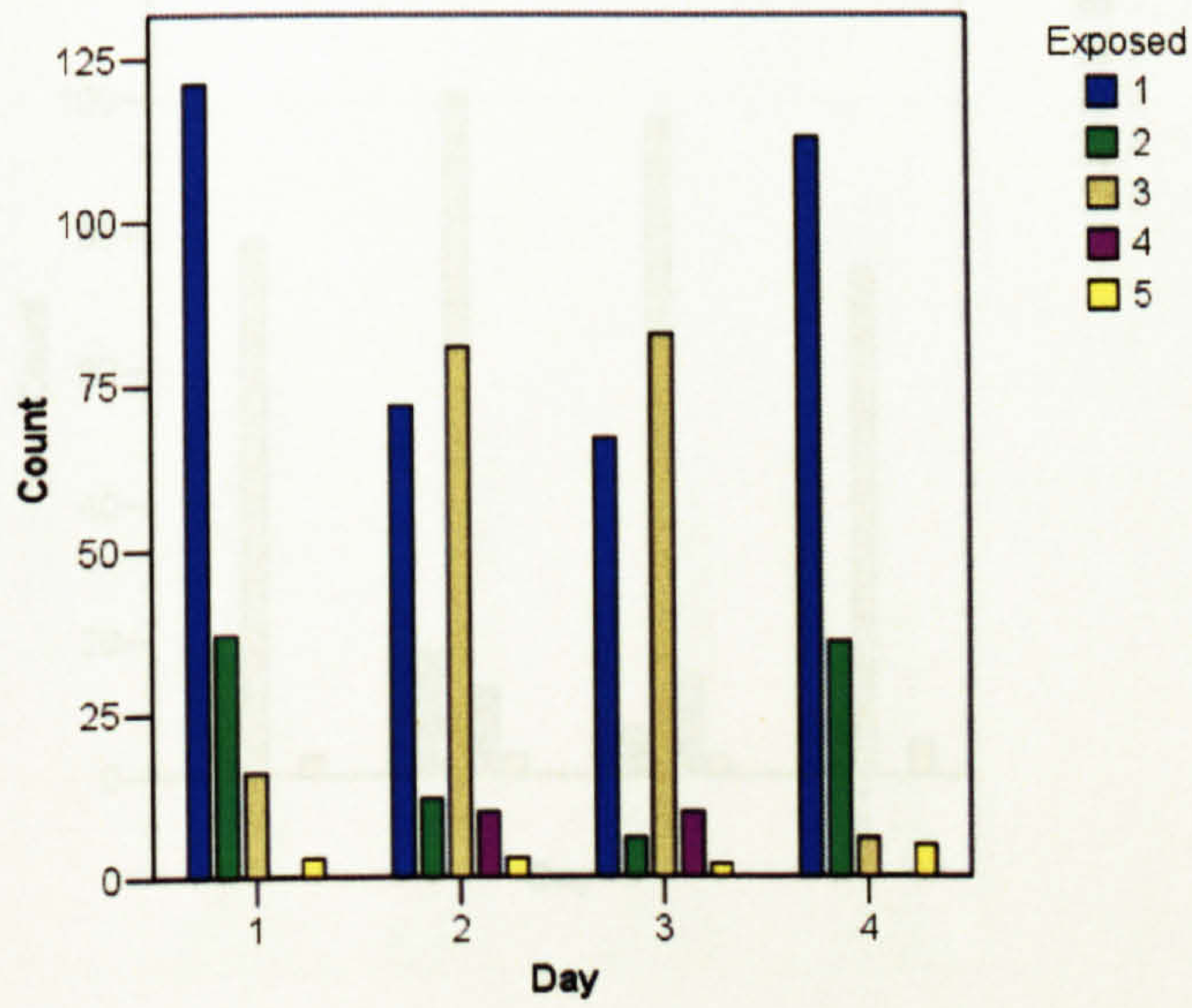


No significant effects of day on 'alertness', the ordinal form of *behaviour*, nor on categorical measures of *behaviour*, were found. Although a reduction in resting (and concomitant increase in resting alert) is indicated by the graph, this was not significant.

Exposed Removing scores of '5' (other) converts *exposed* to an ordinal scale. A Friedman test of all 4 days was highly significant ($p < .001$) for morning scans, afternoon scans and each day's median. Looking for differences between day 1 and 4, and between days 2 and 3 only, marginal homogeneity tests were NS. Differences between all other pairings were highly significant at $p < .001$, box days being less exposed than the baseline and deprivation days (Fig. 4.35). Looking for differences between days using McNemar-Bowker tests had the same results.

Exposedness changes over days, between box and non-box days. Many cats use boxes when they are present, which makes the cats less exposed. Approximately half of the cats have an exposed score of '3' (head not visible) at any one time, with nearly all the remaining cats being completely exposed (score '1').

Figure 4.35 The number of scan tests in which each class of *exposed* was performed, split by day (pooled data from morning and afternoon scans at all 3 shelters, legend in section 4.2.7). Scorings of 2 are nearly all in Axehayes, and appear to occur at the expense of score 1.

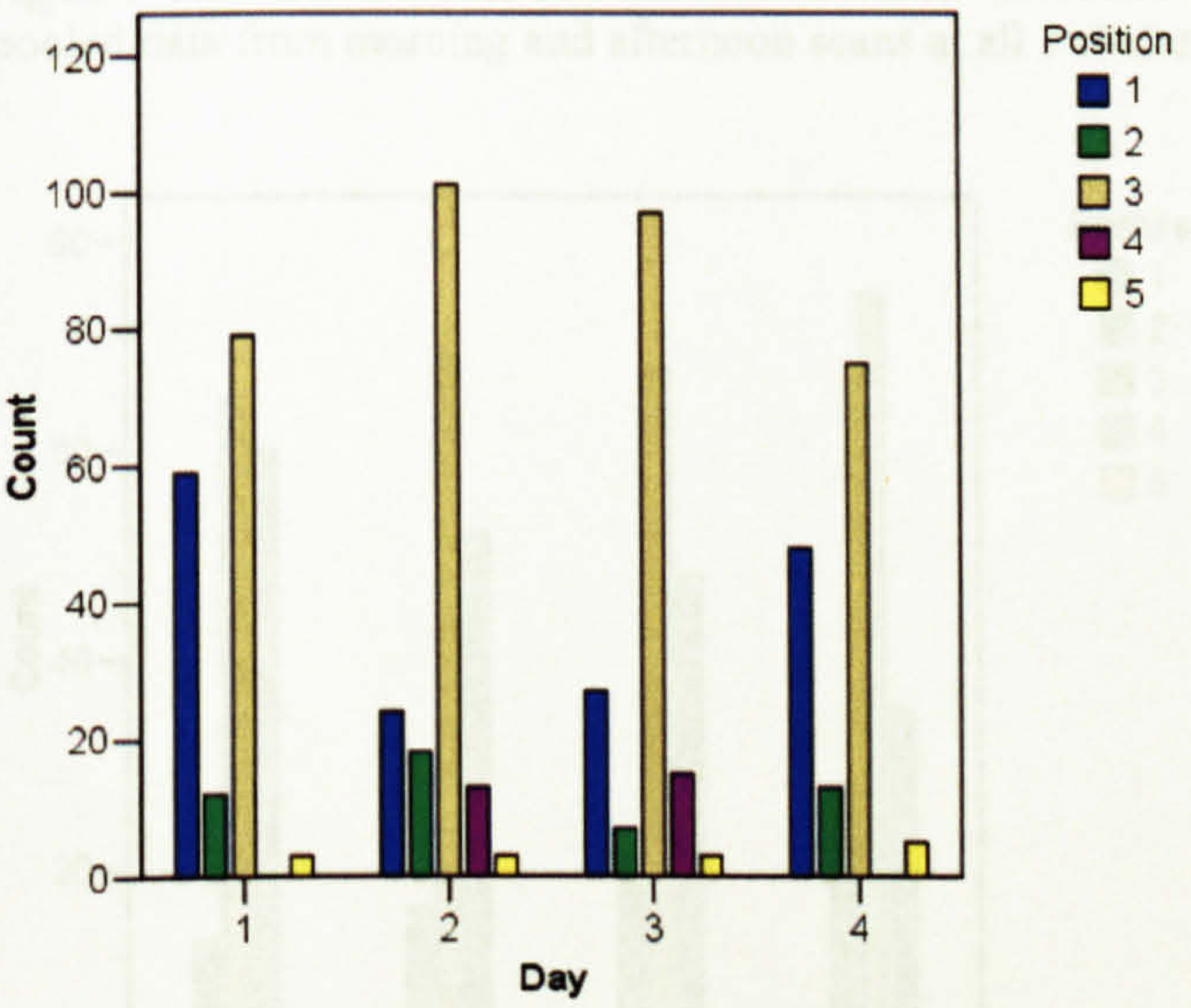


Position

Position for 'in' cats and 'out' cats was analysed separately. For 'out' cats, pairs of days were compared with McNemar-Bowker tests, and were all NS, though the small N (typically N = 10 or less) gave these tests low power. For 'in' cats, morning scans only, d1 v d2 was p = .042, d1 v d3 p = .008, d1 v d4 p = .072, d2 v d3 p = .019, d2 v d4 p = .093 and d3 v d4 p = .033. For afternoon scans, d1 v d2 p = .001, d1 v d3 p = .038, d1 v d4 p = .406, d2 v d3 p = .088, d2 v d4 p = .005, d3 v d4 p = .075. All significant comparisons were between day 1 and days 2 and 3, and between day 4 and days 2 and 3.

Similar changes across days appeared to be causing significance in both morning and afternoon scan samples (Fig. 4.36). For the significant comparisons between day 1 and days 2 and 3, cats appeared to be going from position '1' (front of cage) to position '3' (back of cage, in box/bed area) and to a lesser extent, position '4' (on top of box) in afternoon scans only. For comparisons between day 2 and 4, and between day 3 and 4, the reverse is true – there is a decrease in position '3' and an increase in '1'.

Figure 4.36 The number of scan tests in which each class of *position* was performed, split by day, for cats inside the pods only (pooled data from morning and afternoon scans at all 3 shelters, legend in section 4.2.7)

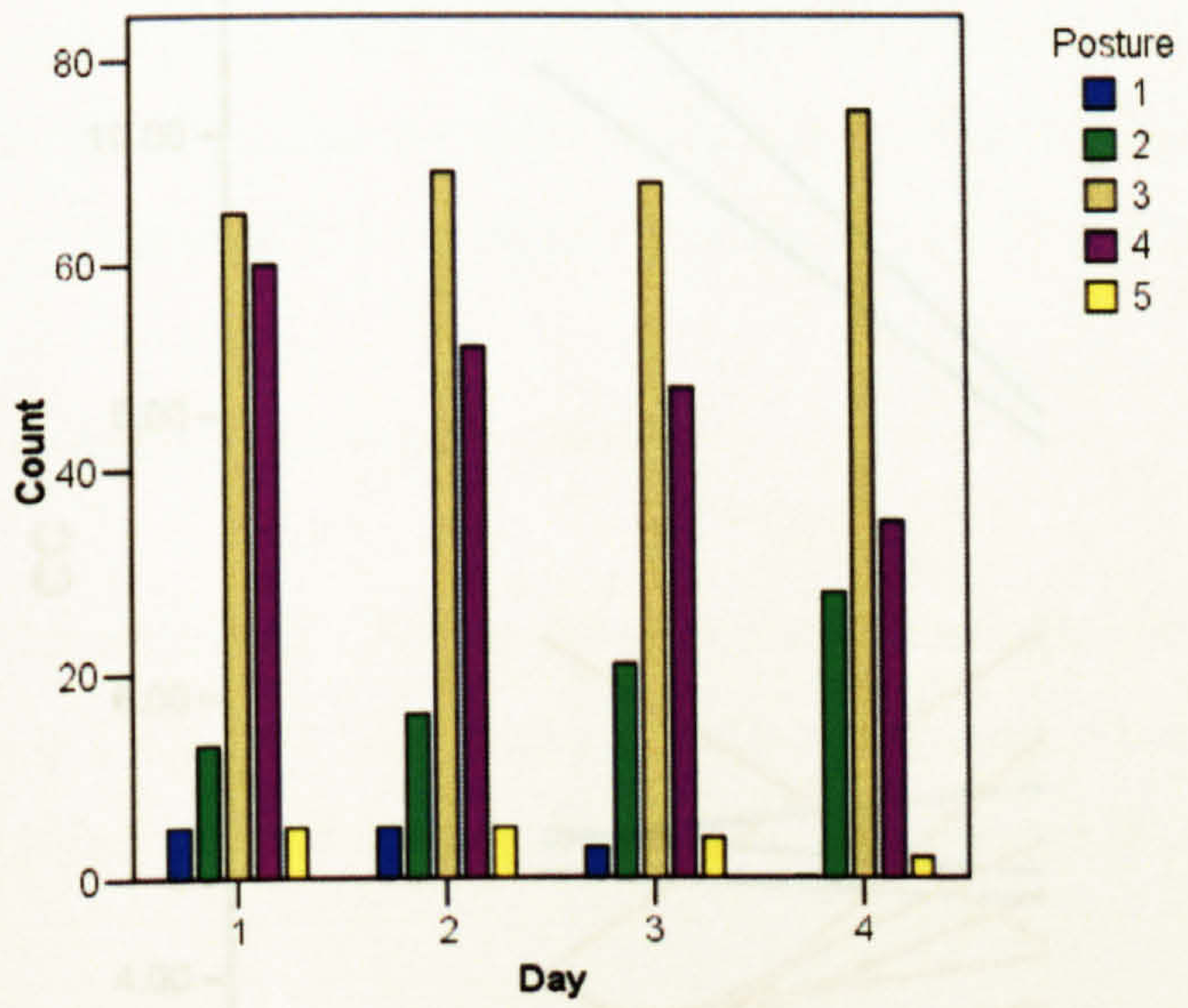


The same trend was not seen for comparison between day 1 and day 4. The significant comparison between d2 and d3 (morning scan) showed a decrease in position '1' and an increase in '4', possibly due to cats becoming more used to the box. The afternoon scan which was close to significance showed the same trend.

No difference in *position* over days for cats when outside was found, though the sample size was quite small (mean N per scan = 10). For inside cats, cats seemed to go from position 1 (at the front of the cage, exposed), to position 3 (in the box) when the box was present, and back again once it was taken away. Boxes do therefore encourage cats to go to the back of the cage. Using the box as a perch appeared to take a little time – cats used it in this way more on day 3 than day 2. This is unlikely to be due to cats needing time to 'discover' this use of the box – cats are used to jumping up and down from objects, and often prefer to be above ground level (Rochlitz 2000b). It may be that it the novelty value of having a hiding place encourages cats to use it as that before using it as a perch.

Posture Class '6', lying ventral on all 4 paws, was recoded as class '3', lying ventral to simplify interpretations. Pairs of days were compared with McNemar-Bowker tests. All morning scans were $p > 0.1$ apart from d1 v d4 which had $p= .049$ (increase in '2', decrease in '4' from d1 to d4) (Fig. 4.37). All afternoon scans were $p > 0.1$. Given the multiplicity, all results were NS.

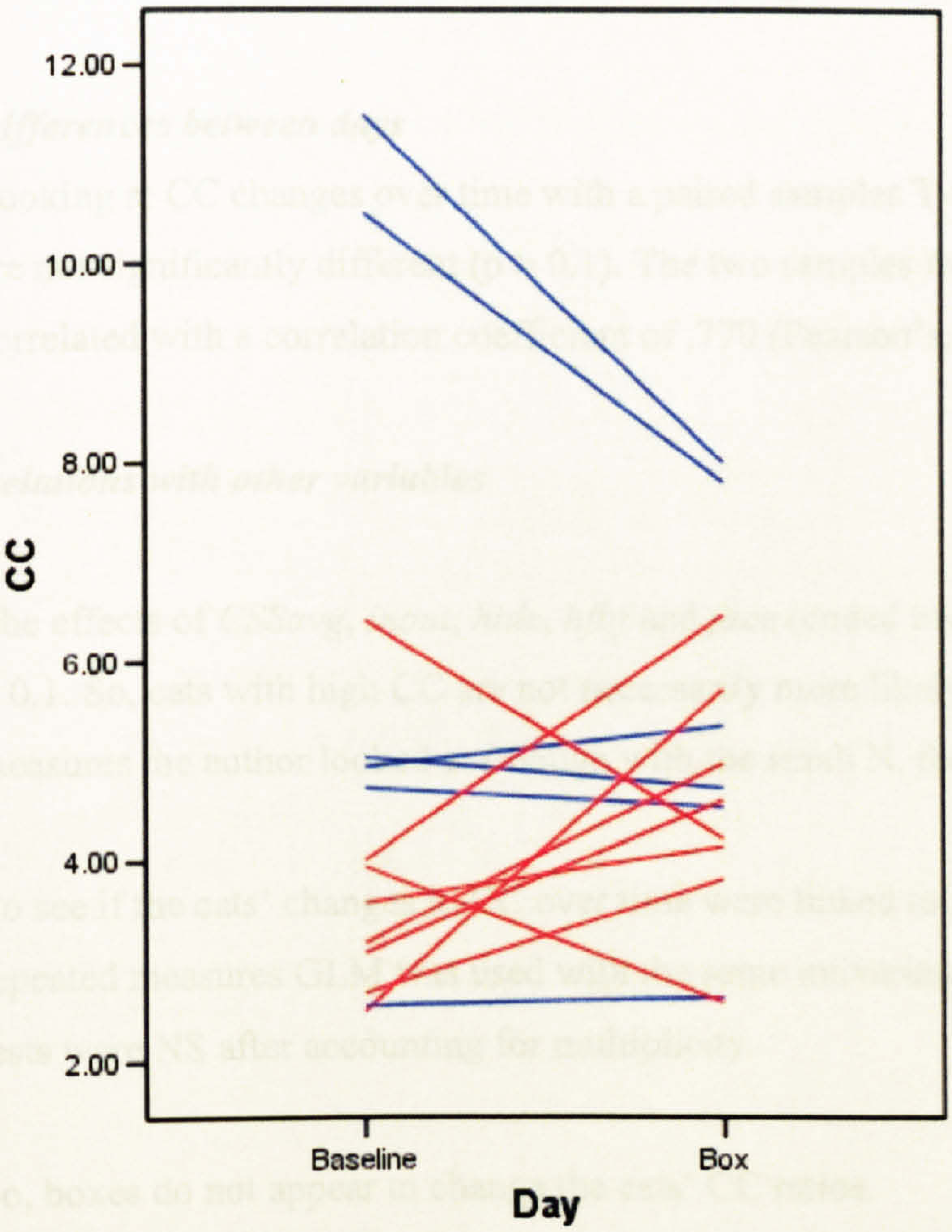
Figure 4.37 The number of scan tests in which each class of *posture* was performed, split by day (pooled data from morning and afternoon scans at all 3 shelters, legend in section 4.2.7)



4.3.5 Cortisol

The raw data for baseline and box CC is shown below (Fig. 4.38).

Figure 4.38 CC ratios on baseline and box days. NCC cats in blue, Axehayes in red.



Differences between shelters

Although independent samples T-tests on baseline CC (NCC mean 6.55, Axehayes mean 3.64), and on box CC (NCC mean 5.52, Axehayes mean 4.85) were NS at p > 0.1, the change in CC (baseline minus box CC, NCC mean 1.03, Axehayes mean -1.21) was significantly different between shelters (p = .036). NCC cats either had no real change, or a decrease, while Axehayes cats generally increased between baseline and box. This decrease is largely fuelled by the two cats which had baseline CC ratios around 10, far higher than the other cats. Their drop in CC is concomitantly greater. If these two are removed as outliers, the significant relationship no longer exists ($p > 0.1$). The author believes that the difference is not due to shelter differences, merely chance that both these two cats were at NCC. If the change is restated as a proportion of the baseline CC (i.e. baseline divided by box) and retested, it is NS at $p > 0.1$. In conclusion, there is no evidence for a significant difference between shelters.

Differences between days

Looking at CC changes over time with a paired samples T-test, baseline and box CC are not significantly different ($p > 0.1$). The two samples from each cat were highly correlated with a correlation coefficient of .770 (Pearson's, $p = .002$).

Relations with other variables

The effects of *CSSavg*, *inout*, *hide*, *hfbf* and *face* (coded bivariately) were all NS at $p > 0.1$. So, cats with high CC are not necessarily more likely to perform any of the measures the author looked at (though with the small N, these tests had low power).

To see if the cats' changes in CC over time were linked to any other variables, repeated measures GLM was used with the same univariate tests as above. Again, all tests were NS after accounting for multiplicity.

So, boxes do not appear to change the cats' CC ratios.

4.3.6 Baseline CSS ("stress")

Baseline CSS ("stress") and CSS

The initial model was:

CSSday 2,3,4 = shelter + sex + stray + sex*stray + *stress*

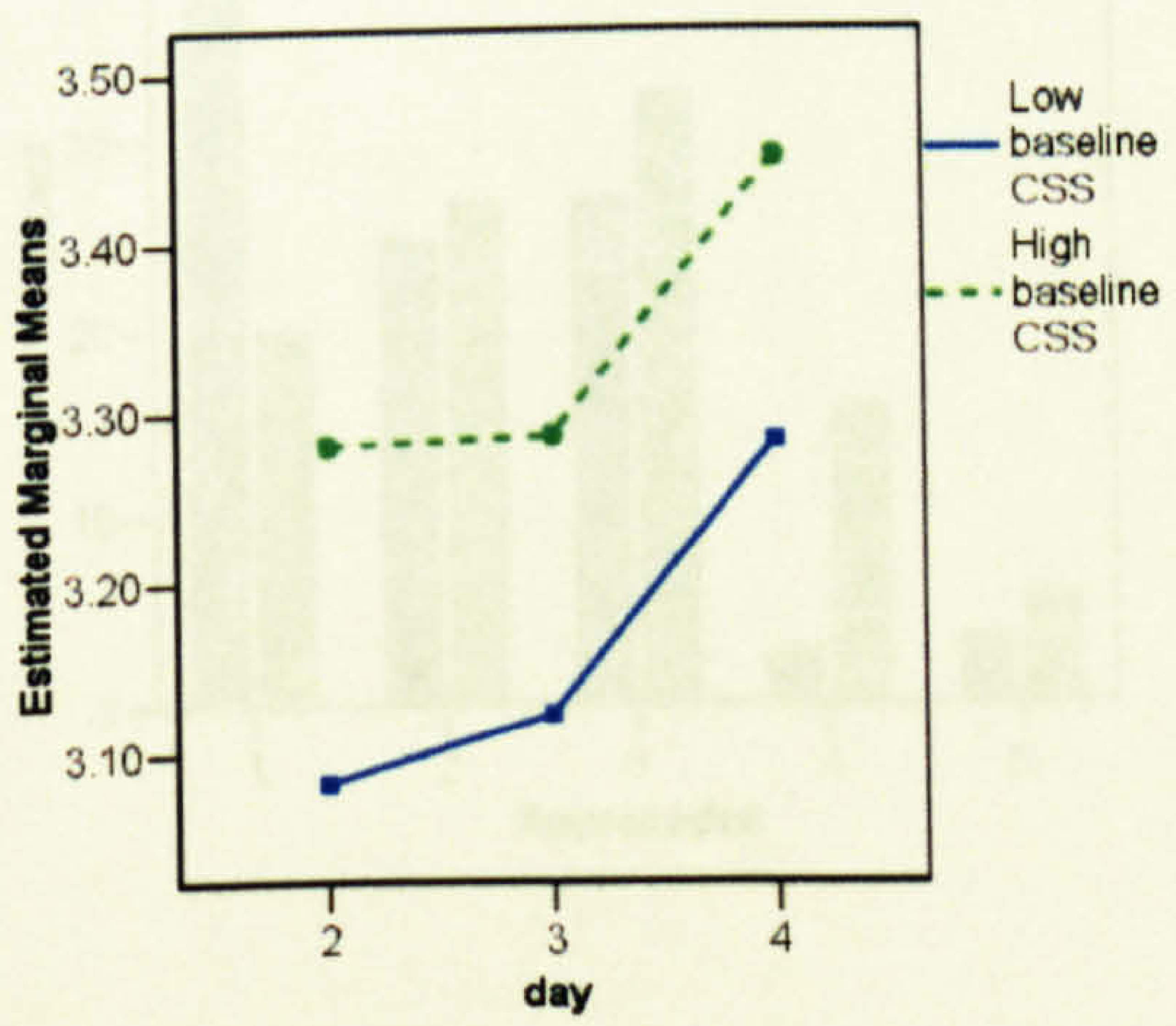
For analysis of CSS only, Bath data was removed from the model, as indicated in Section 4.3.2. The p-values are in Table 4.21. The graphs of marginal means of CSS split by shelter, sex and stray were very similar to those in section 4.3.2, which indicated that the inclusion of stress had not changed the relationship between these variables and stress. A graph of marginal means of CSS split by *stress* is in Figure 4.39.

Table 4.21 p-values for terms in the GLM equation CSSday2,3,4 = shelter + sex + stray + sex*stray + *stress*, no Bath data. N = 71

Variable	Multivariate tests	Within-subjects effects
Day	.004	.003
Day*Shelter	.324	.321
Day*Sex	.116	.104
Day*Stray	.686	.659
Day*Sex*Stray	.232	.229
Day**stress*	.922	.927

Variable	Between-subjects effects
Shelter	.181
Sex	.053
Stray	.612
Sex*Stray	.070
Stress	.031

Figure 4.39 Estimated daily marginal means for CSS, split by *stress*



Stress did not have a significant effect across days (Table 4.21: within-subjects $p = .927$), though was significant between cats ($p = .031$), having a near constant effect from day to day with *low stress* cats remaining at a low CSS and *high stress* cats remaining high (Fig. 4.39). There is no evidence that *high stress* cats had a greater decrease in CSS on box days. To check for an interaction between *stress* and either sex or stray, the following model was also tested:

CSSd2,3,4 = shelter + sex + stray + sex*stray + *stress* + *stress**shelter + *stress**sex + *stress**stray

None of the interaction terms with stress were significant in multivariate, within-subjects or between-subjects tests ($p > 0.1$), confirming that stress has a solely additive effect.

Baseline CSS ("stress") and Approach test

Combining data from all four days together, *low stress* cats tended to have lower values for *apprecoded, friendliness* and *CSSupdown* than *high stress* cats, indicating that *high stress* cats had a higher aversion to the observer (Figures 4.40, 4.41 and 4.42).

Figure 4.40 Percentage of cats exhibiting each class of *apprecoded*, split by *stress* (pooled data from all approach tests, legend in section 4.2.7)

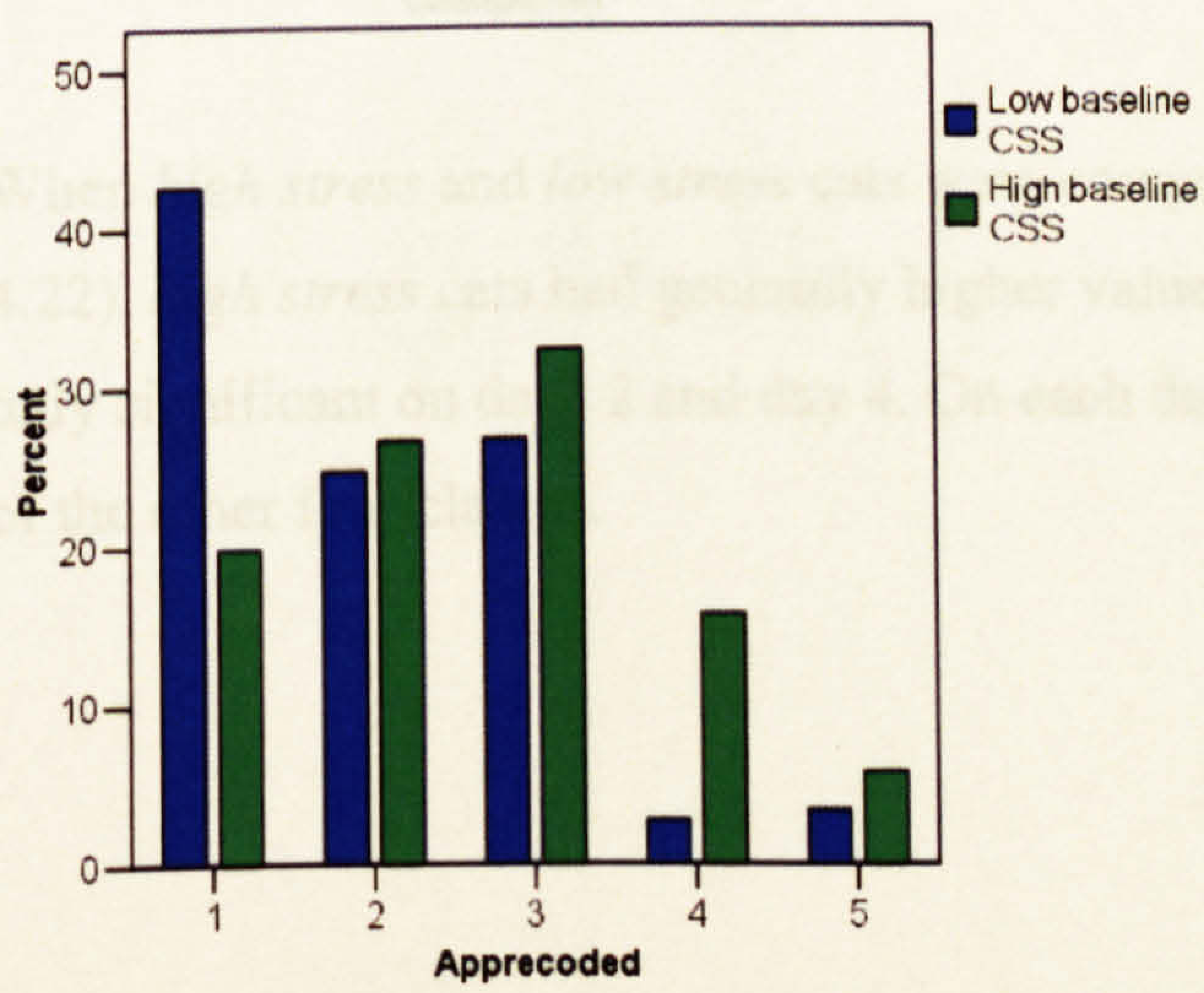


Figure 4.41 Percentage of cats exhibiting each class of *friendliness*, split by *stress* (pooled data from all approach tests, legend in section 4.2.7)

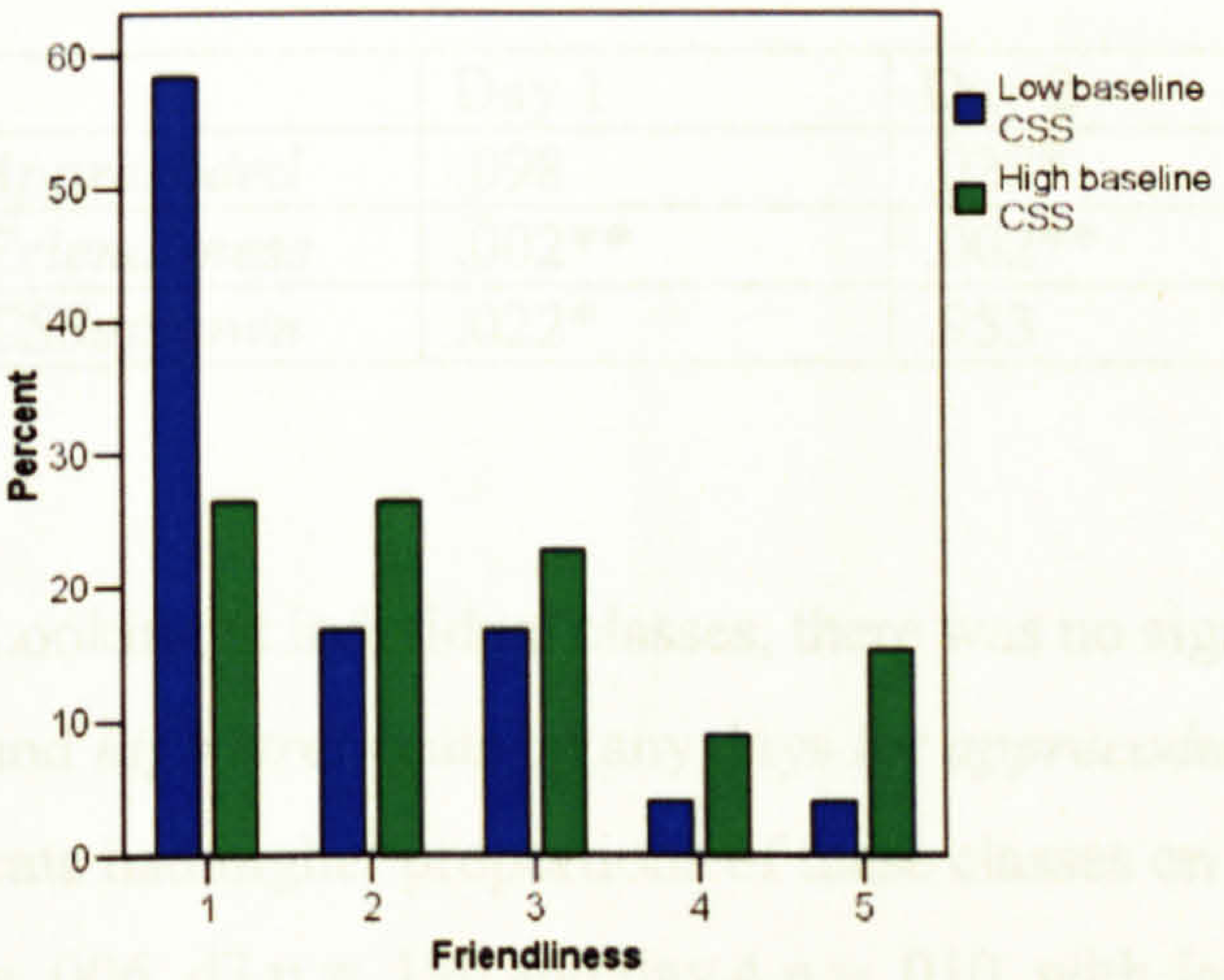
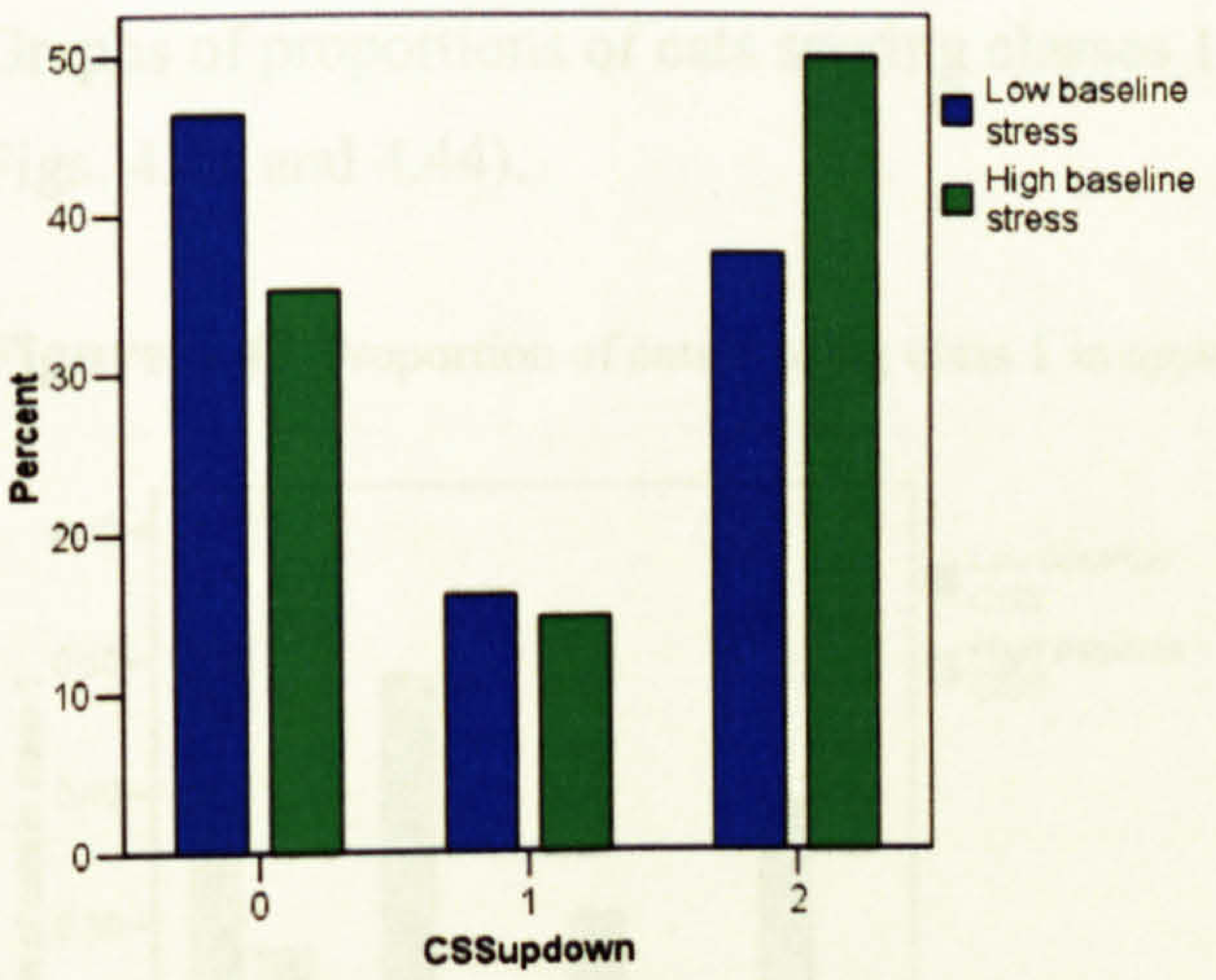


Figure 4.42 Percentage of cats exhibiting each class of *CSSupdown*, split by *stress* (pooled data from all approach tests, legend in section 4.2.7).



When *high stress* and *low stress* cats were compared for each day separately (Table 4.22), *high stress* cats had generally higher values for *apprecoded*, though this was only significant on days 2 and day 4. On each day, they scored fewer class 1 and more of the other four classes.

Table 4.22 p-values for differences between *high stress* and *low stress* groups, on each day, for variables *apprecoded* (Chi-sq), *friendliness* and *CSSupdown* (both Mann-Whitney). * = p < .05, **= p<.01

	Day 1	Day 2	Day 3	Day 4
Apprecoded	.098	.028*	.204	.018*
Friendliness	.002**	.002**	.007**	.004**
CSSupdown	.022*	.953	.347	.140

Looking at individual classes, there was no significant difference between *low stress* and *high stress* cats on any days for *apprecoded* classes 2, 3 or 5, although *high stress* cats had higher proportions of these classes on all days. For class 1, d1 p = .030, d2 p = .006, d3 p = .190 and day 4 p = .010, with *low stress* cats having a higher proportion of class '1'. For class 4, d1 p = .072, d2 p = .176, d3 p = .132 and d4 p = .008, with *high stress* cats having a higher proportion of class 4.

Graphs of proportions of cats scoring classes 1 and 4, split by day and *stress* are in Figs. 4.43 and 4.44).

Figure 4.43 Proportion of cats scoring class 1 in *apprecoded*, split by day and *stress*

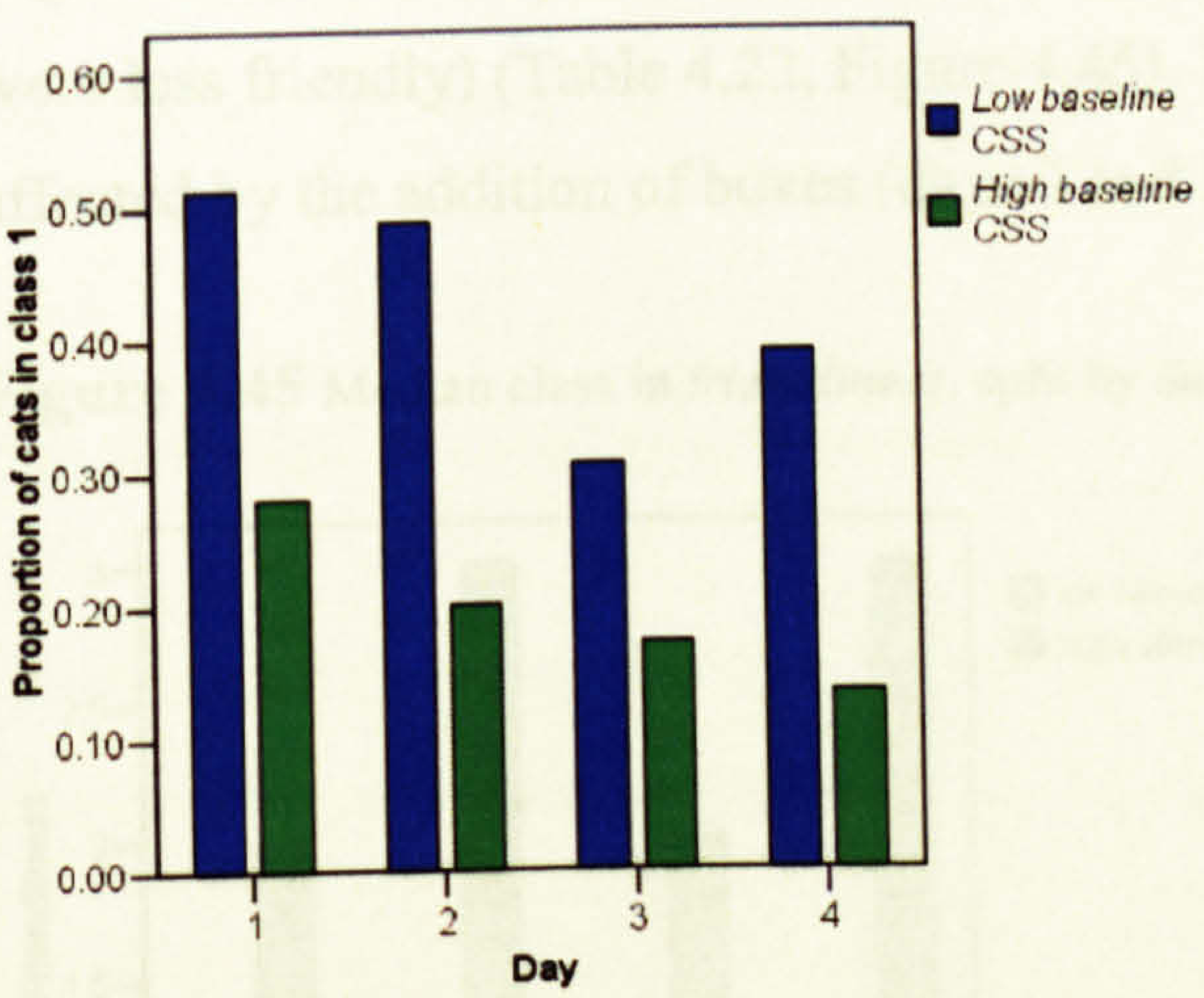
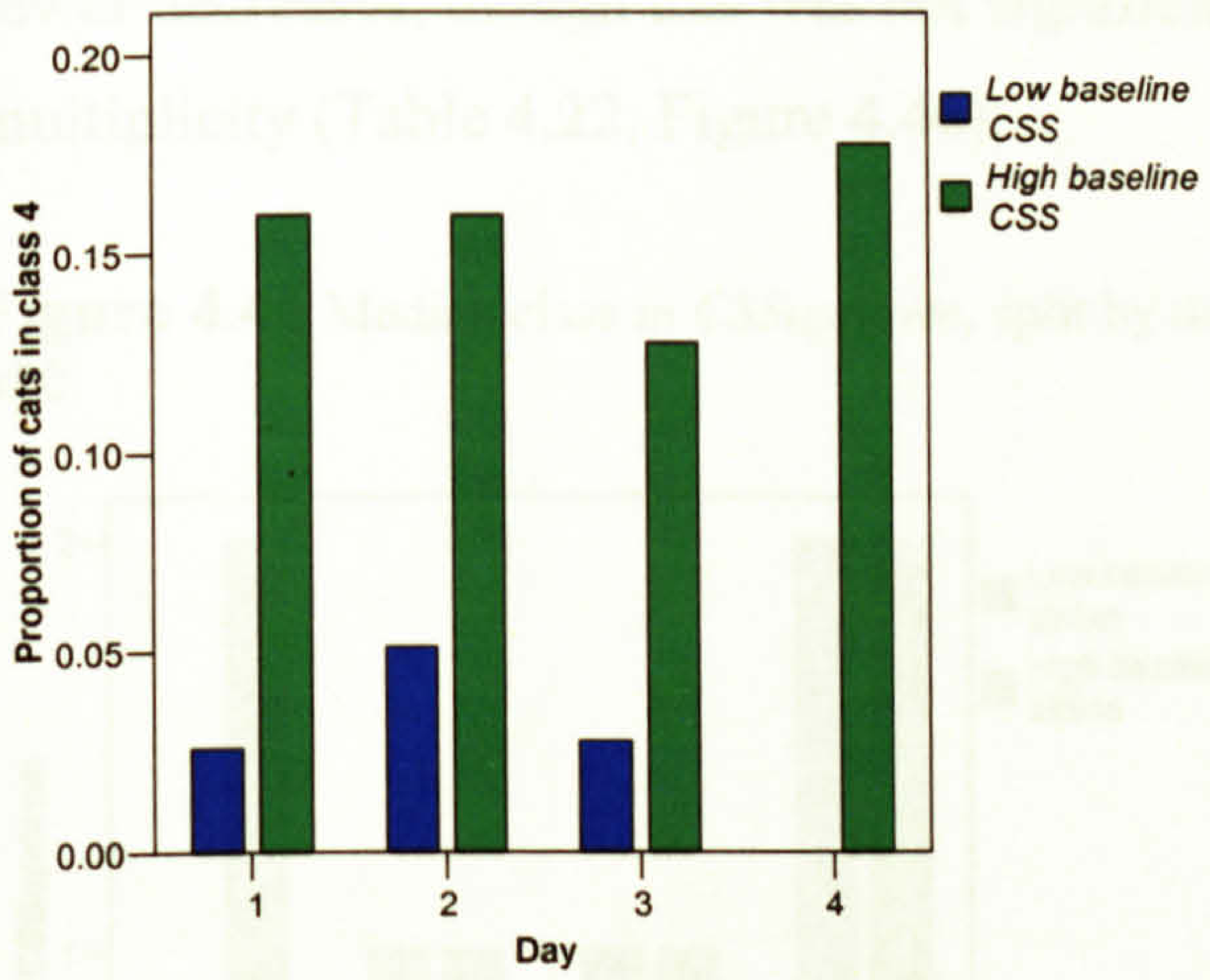


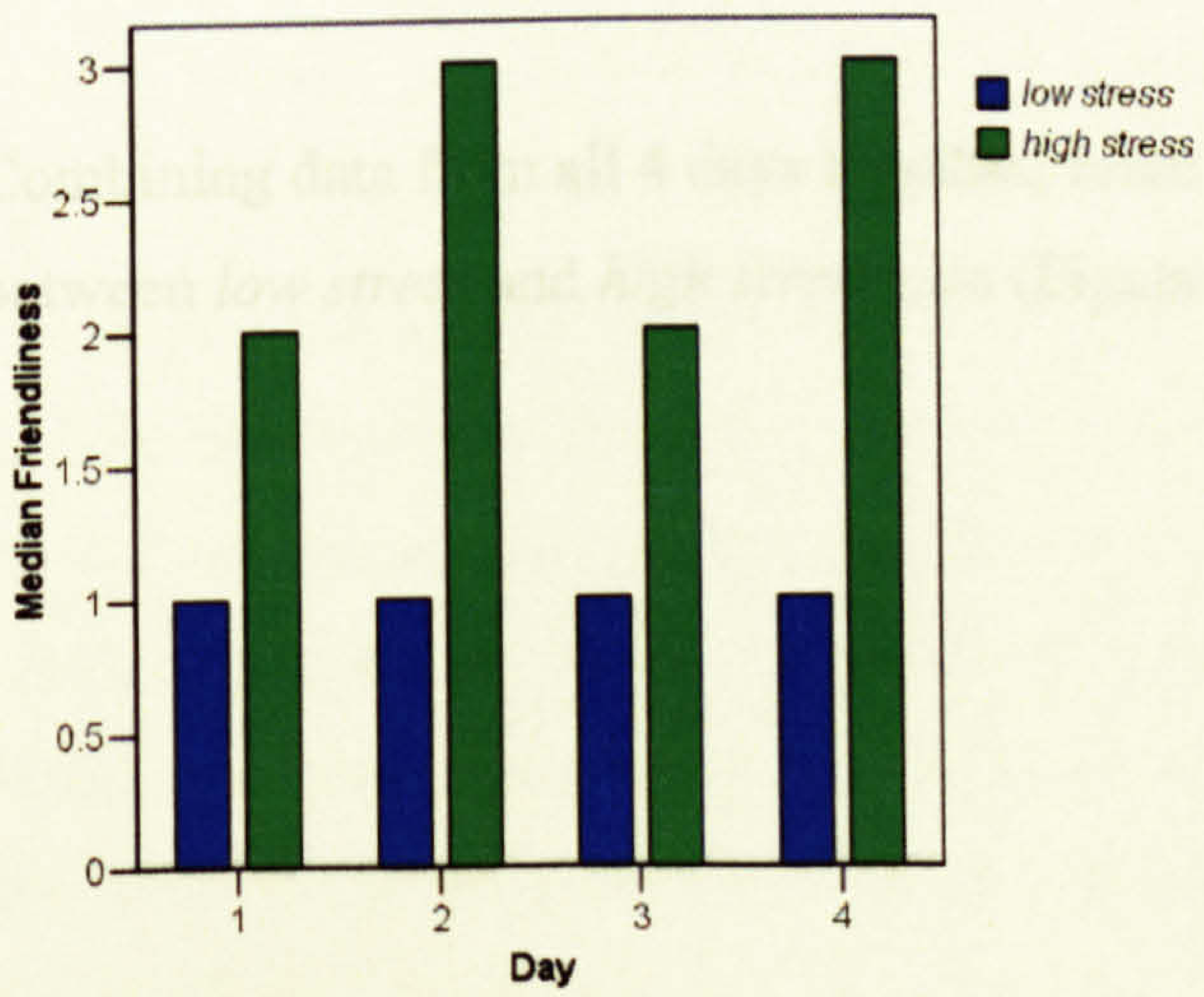
Figure 4.44 Proportion of cats scoring class 4 in *apprecoded*, split by day and *stress*



Although *low stress* cats are more likely to be placed in class 1 than *high stress* cats, there doesn't appear to be an effect of box on either *low stress* or *high stress* cats (this was checked across all 4 days with Cochran's Q, NS at $p > 0.1$ for both groups). Class 4 is similar – although *high stress* cats are more likely to be in class 4, the effect of box is not significantly different for the two groups ($p > 0.1$ for both groups).

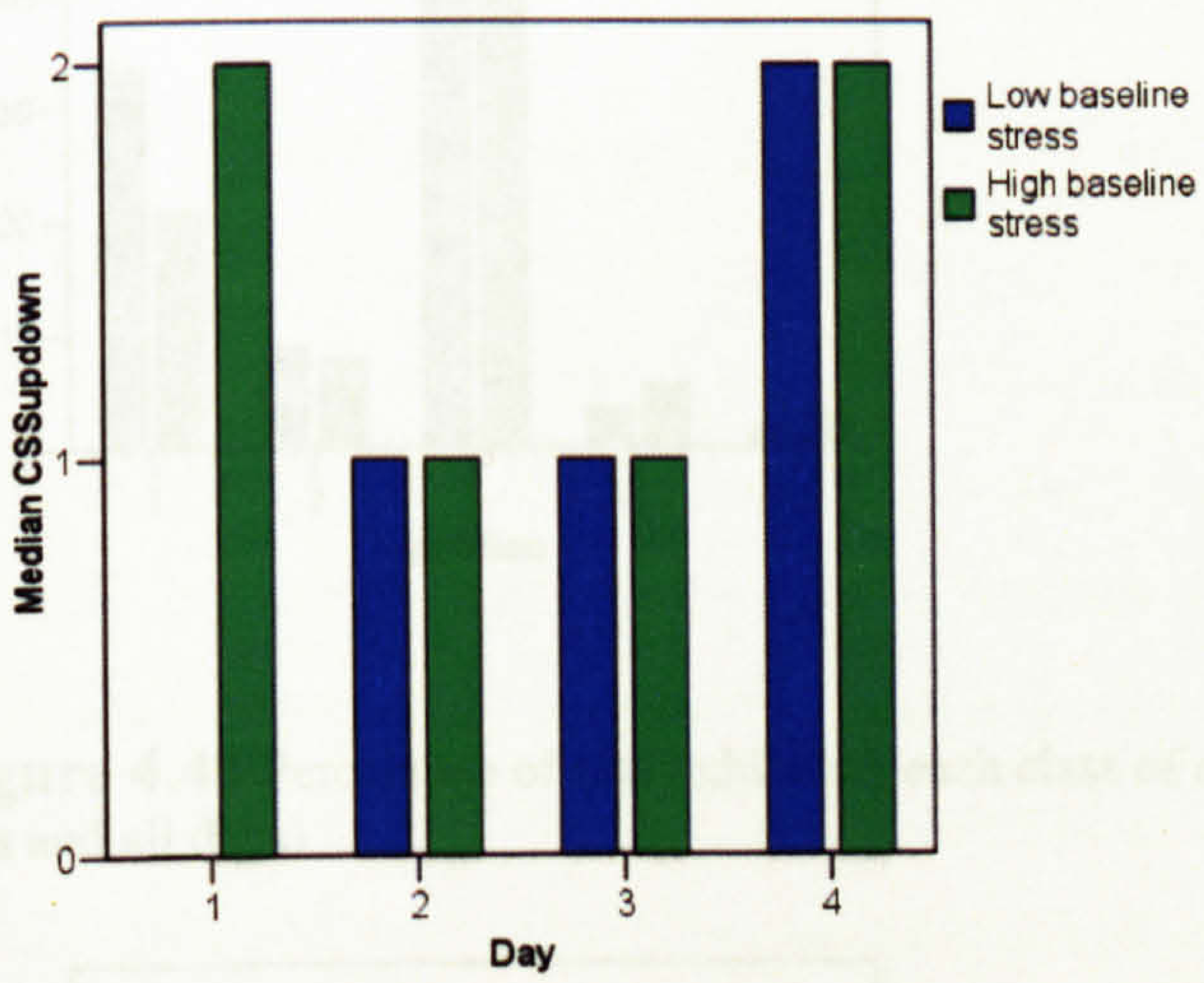
High stress cats had significantly higher scores for *friendliness* on every day (i.e. they were less friendly) (Table 4.22, Figure 4.45). Medians for *friendliness* were similarly affected by the addition of boxes (days 2 and 3), and their withdrawal.

Figure 4.45 Median class in *friendliness*, split by day and *stress*



High stress cats had generally more increases in CSS following the approach test and fewer decreases, though this was not significant on any day after accounting for multiplicity (Table 4.22, Figure 4.46).

Figure 4.46 Median class in *CSSupdown*, split by day and *stress* d1 low baseline stress had a median of 0.



In summary, cats with high baseline CSS were less friendly, whether the box was present or not, and somewhat less likely to approach the observer during the first phase of the approach test.

Baseline CSS ("stress") and Scan test

Combining data from all 4 days together, there was little observable difference between *low stress* and *high stress* cats (Figures 4.47 and 4.48).

Figure 4.47 Percentage of cats exhibiting each class of *position*, split by *stress* (pooled data from all cats and all days). *Histogram* of *position* ("In" data only), split by *stress*, data from all cats and scans

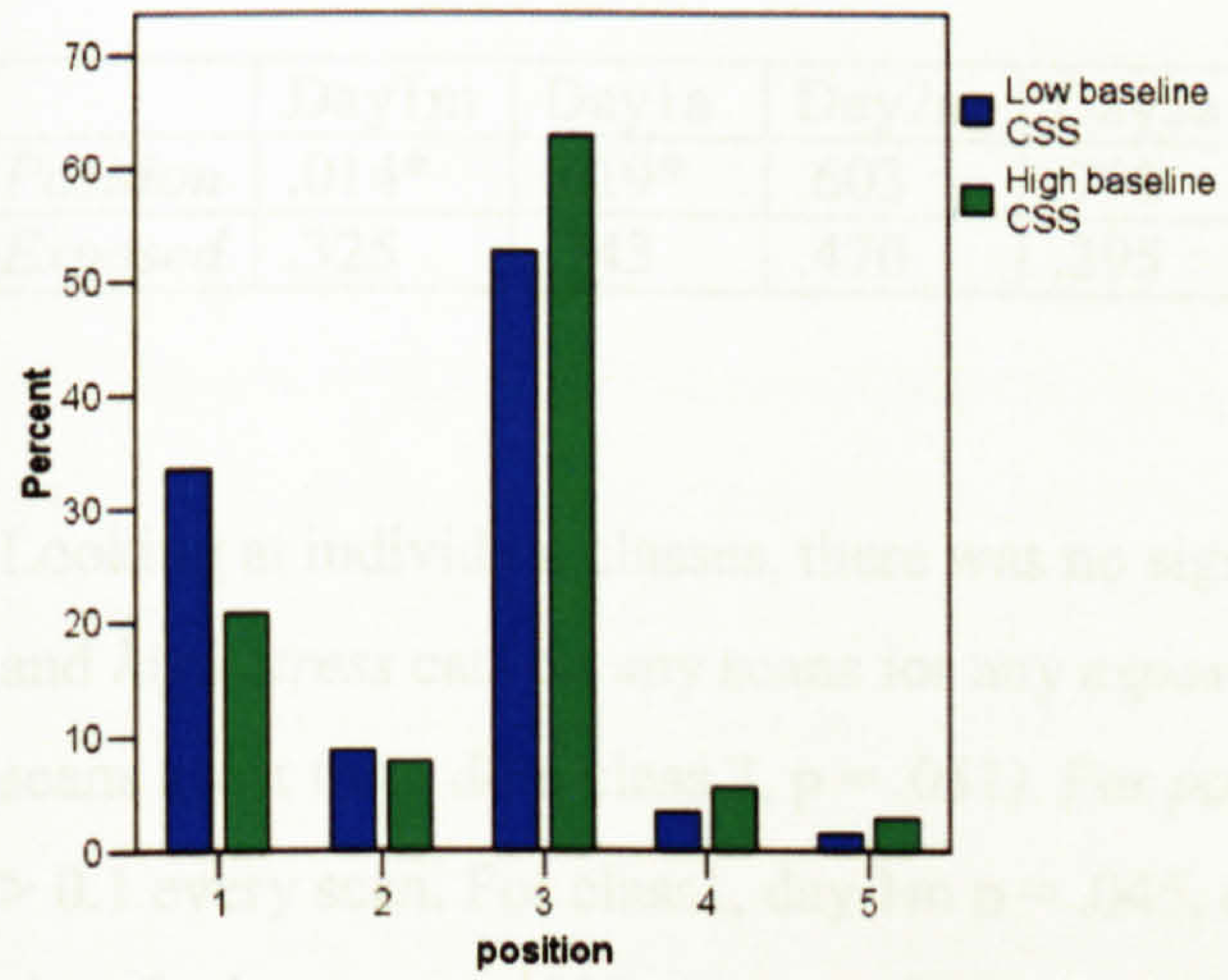
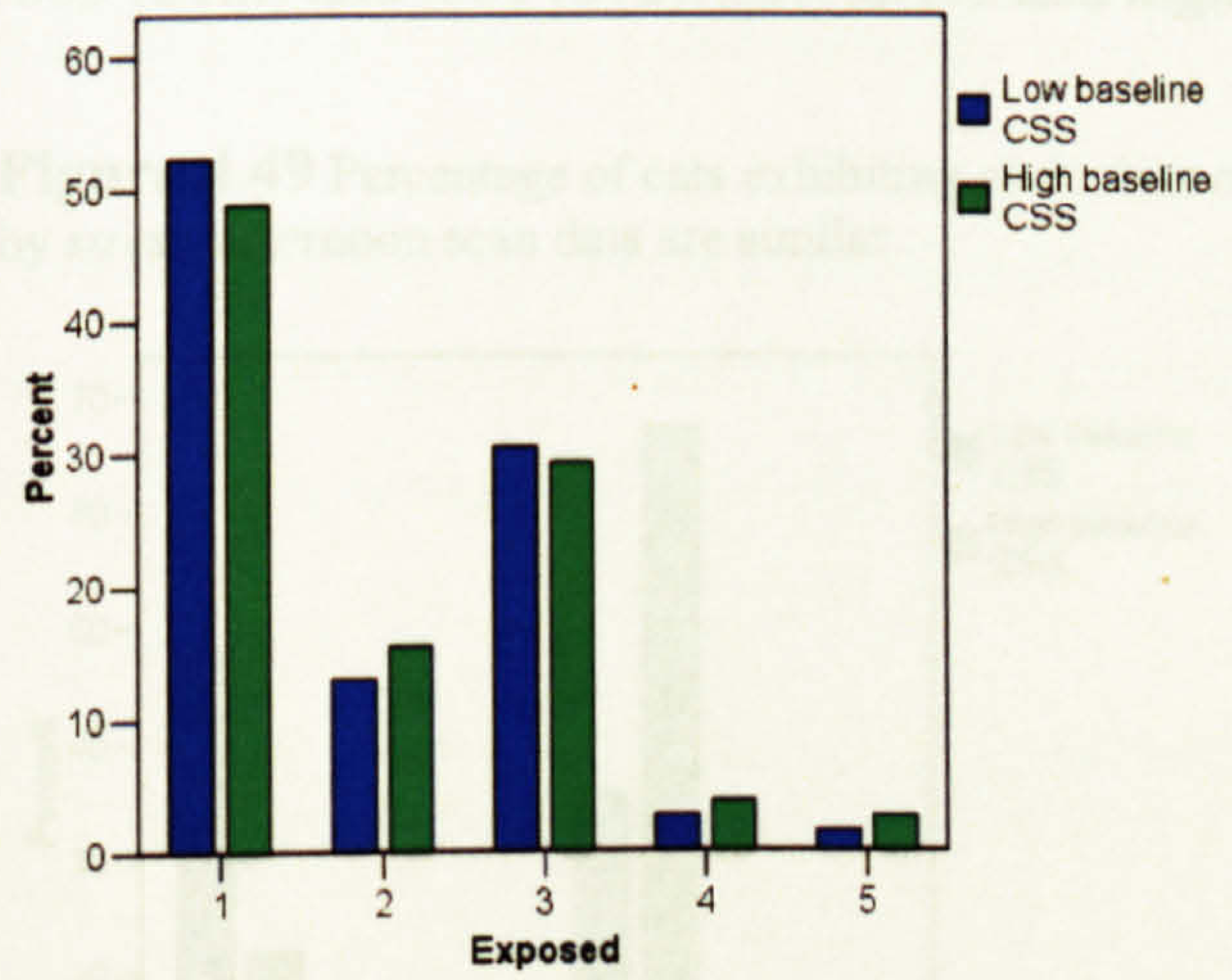


Figure 4.48 Percentage of cats exhibiting each class of *exposed*, split by *stress* (pooled data from all cats and all days)



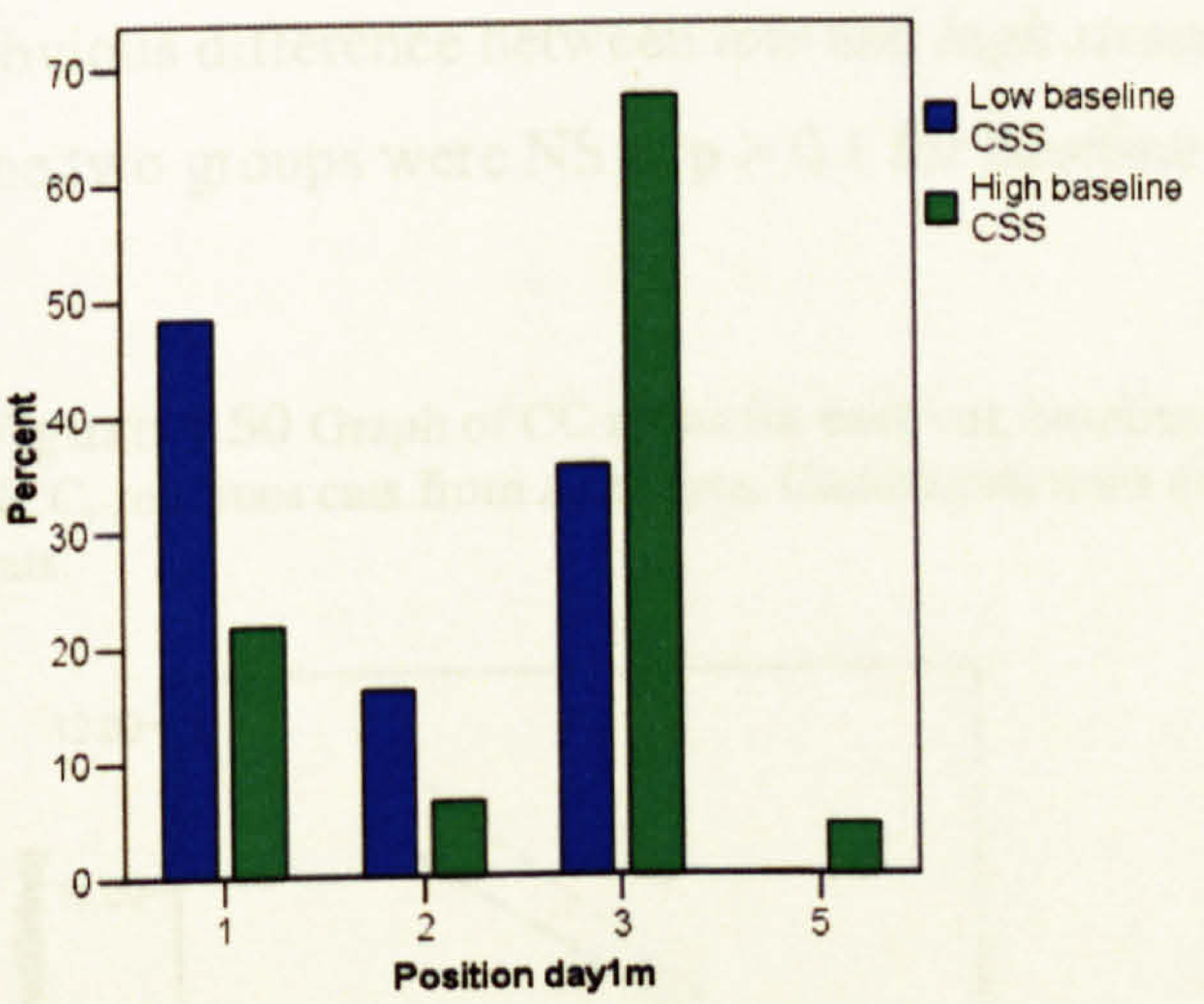
When *high stress* and *low stress* were compared for each day separately (Table 4.23), there was no significant difference between these groups either in *exposed*, or in *position* once multiplicity is taken into account. The low p-values for *position* day 1 (in both cases, cats showed less 1 and more 3) may have been due to non-independence with *stress* (since CSS and scan data are recorded at the same time). It was not due to the large proportion of Bath data in *high stress*, as Bath data was similar to that of other shelters.

Table 4.23 p-values for differences between *high stress* and *low stress* groups, on each set of scans, for variables *position* (Chi-sq, 'in' cats only) and *exposed* (Mann-Whitney, recoded as ordinal data). m=morning, a=afternoon. * = p<.05, ** = p<.01

	Day1m	Day1a	Day2m	Day2a	Day3m	Day3a	Day4m	Day4a
Position	.014*	.019*	.603	.790	.495	.723	.985	.793
Exposed	.325	.243	.470	.395	.737	.812	.503	.852

Looking at individual classes, there was no significant difference between *low stress* and *high stress* cats on any scans for any *exposed* classes (all NS at p > 0.1 for all scans apart from d4m class 3, p = .051). For *position*, classes 2, 4 and 5 were NS at p > 0.1 every scan. For class1, day 1m p = .045, day 1a p = .002, rest NS at p > 0.1. For class 3, day 1m p = .002, d1a p = .024, rest NS at p > 0.1. As above, day 1 results were due to *low stress* cats having higher levels of '1' and lower levels of '3'. This is not the result of the Bath data, since removal of Bath data decreased the p-value to p = .005 (d1m) and .001 (d1a). *Low stress* and *high stress* position data are in Figure 4.49.

Figure 4.49 Percentage of cats exhibiting each class of *position* on the morning scan of day 1, split by *stress*, afternoon scan data are similar



As above, this day 1 difference could be due to *position* and CSS being recorded at the same time. Alternatively, it could be a real relationship, that shows up only on day 1 because boxes reduce the effect (drawing many cats to position '3'), and the cats have become used to the observer by day 4. Though it is not possible to test this hypothesis explicitly, day 4 data was analysed the same way day 1 data was, and split by average CSS on day 4.

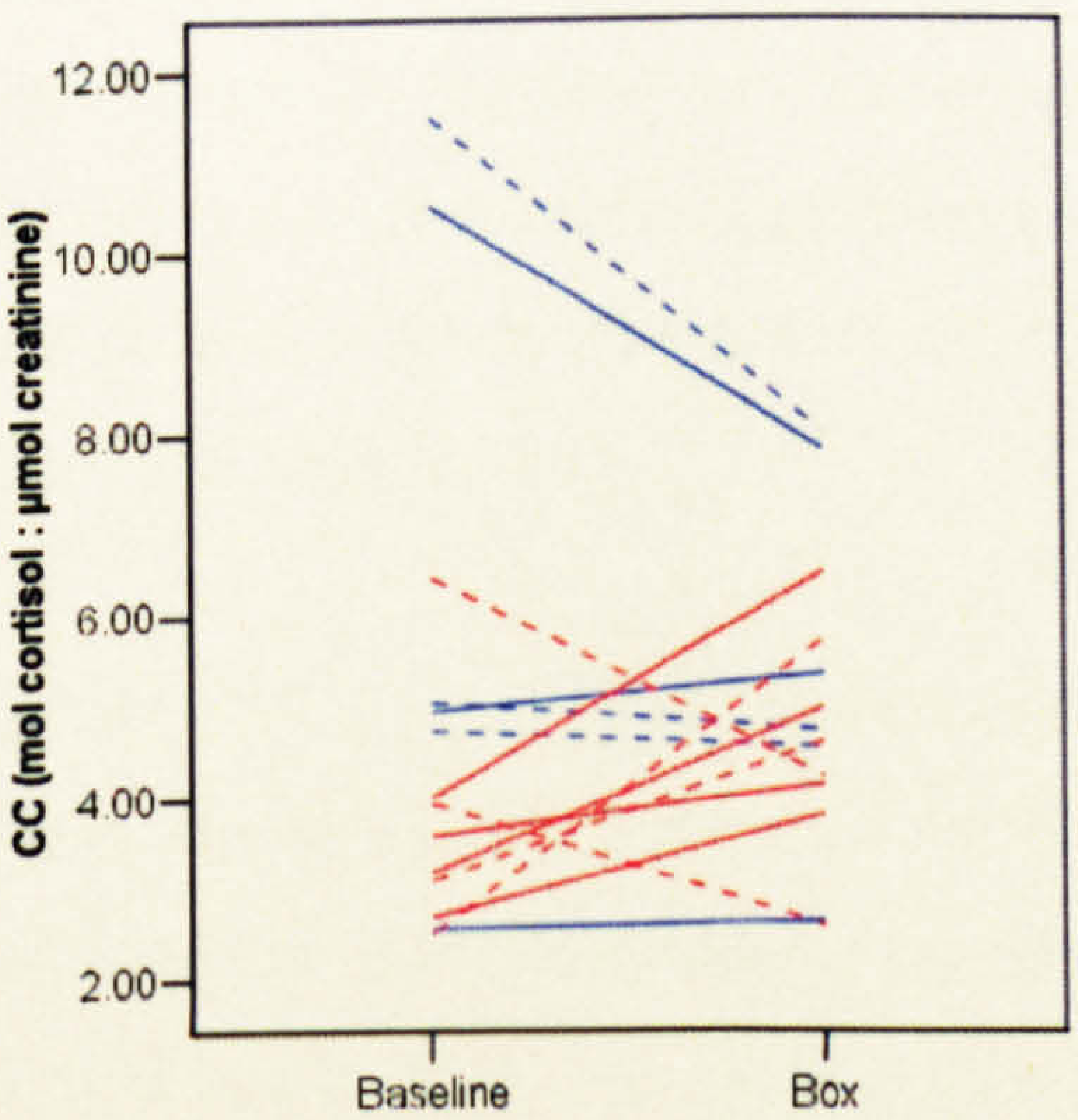
Day 4 data was split by average CSS on day 4. Since CSS was elevated on day 4 compared to day 1, the cut-off point was chosen as CSS of 3.25 (as opposed 3.0 for *stress*) which split the cats into: 3.25 or below, N = 41, 3.5 or above, N = 39. Having labelled data by '*d4stress*', day 4 *position* had *low d4stress* cats compared against *high d4stress* cats, as above. A chi-sq test on d4m was significant at p = .007, while d4a had p = .027. As before, *low d4stress* had higher levels of position 1 and low levels of position 3 when compared to *high d4stress*. Similar results were found with *exposed*, with d4m p = .076, d4a p = .022. This suggests that the significant day 1 values are due to non-independence with CSS, so can be ignored.

In conclusion, there are no significant differences between *low stress* and *high stress* cats in approach test variables.

Baseline CSS ("stress") and cortisol

Baseline and Box CC ratios classed by *stress* are below (Figure 4.50). There is no obvious difference between *low* and *high stress* cats. Independent samples T-tests of the two groups were NS at p > 0.1 for baseline CC, box CC and box-base CC.

Figure 4.50 Graph of CC ratios for each cat, baseline and box samples. Blue lines are cats from NCC, red lines cats from Axehayes. Continuous lines are *low stress* cats, dashed lines are *high stress* cats.



The initial repeated measures GLM equation was:

CCbase, box = *stress* + shelter + *stress**shelter

Multivariate, within-subjects and between subjects tests were all NS at $p > 0.1$ apart from CC*stressed, multivariate test $p = .082$. Removing the shelter and stress*shelter terms to leave stress as the only factor (x-variable) and retesting, all tests were NS at $p > 0.1$. All tests remained NS with the two high CC outliers removed. So, there is no significant difference between *high-* and *low stress* groups, though the low N gave these tests a low power.

4.4 Discussion

4.4.1 Maintenance

Although *urinate* was unaffected by the experimental treatment, *feed* did differ between days: the incidence of not feeding during the day appeared to be higher on the first two days. Not feeding during the day may simply be part of the personality of a particular cat, but missing the morning meal entirely when it would normally eat could be a sign of stress. Since most shelters had fewer people on days 1 and 2 than at the weekend (days 3 and 4) it seems unlikely that it was due to the extra stress caused by the public. Since the box days are days 2 and 3, they do not seem a likely cause either.

The lack of *feed*ing might be a reaction to the observer being present: if the author's presence stressed the cats, they might eat less during the day. Also possible is that author's presence calmed the cats; in the observations reported in Chapters 1 and 2 cats frequently ate during approach tests (more so than would be expected by chance, pers obs). Cats have socially facilitated feeding (Bradshaw 1992), and it is possible that the continued presence of a familiar individual encouraged some cats to eat during the day which they would not do otherwise. However, the staff were also frequently present and would have been known to the cats. The author probably interacted for longer each day with the cats than the staff did, but the main difference may have been time of day that the interactions occurred. Most interactions with staff centred around cleaning and providing food in the morning, which is a stressful time for many cats. Interactions with the author were later during the day after that stressor had passed, and this may have been the time that a known human's presence might most facilitate feeding.

Of the 6 NCC cats that did not eat during the day on d1, only one of those was also one of the 8 cats that did not eat during the day on day 2. Since it is not the same cats consistently not feeding during the day, it is difficult to tell whether there are other factors involved. It is possible that some cats didn't eat on day 1 because of the presence of the author as an unfamiliar individual, and that some cats didn't eat on day 2 due to stress caused by the presence of an unfamiliar box.

In conclusion, it is unclear what is driving the change in *feed* over the course of the experiment.

4.4.2 CSS

Although box days had lower CSS than non box days, the tendency was not significant. Day 4 had significantly higher CSS than any of the other days. This may be due to the removal of the box causing stress, or the box masking extra stress caused by the public at the weekend on day 3. However, although NCC had an increase in visitors on day 3 as well as day 4, Axehayes was very quiet on day 3. Looking at the graph of marginal means of CSS split by shelter (Fig. 4.21), Axehayes indeed has the lowest value on day 3 (although day 2 is lower than day 1, with the roughly the same number of people), while NCC has an increase on day 3 to the same level as day 1. This suggests that the public does have an effect on cats' stress. Since day 2 is lower than day 1 for both shelters, even though both shelters had the same number of people, this suggests that boxes also have an effect that may be additive to that of the public. Alternatively, the author's presence may have stressed the cats, which then adapted by day 2. Unfortunately, there is not enough data to adequately test these hypotheses. Day 4 is noticeably higher than the other three days (Fig. 4.20) which suggests removal of the box does cause stress, and indicates that the cats valued its presence.

The CSS in this Chapter is similar to *ScanCSS* from Chapter 3, rather than the videotaped CSS, as it was recorded manually by an observer who had also interacted with the cats. Extrapolating from Chapter 3, this would tend to reduce any 'global' effect of box, due to masking by the cat's responses to the observer. The effect of box might well have been greater had there been no observer present.

The apparent difference in sensitivity of CSS at detecting effects of the addition of the box as compared to its removal may have been a result of the procedure used. Any acute positive effect on CSS of adding the box may have occurred and then waned in the 19 hours between its introduction to the pod and the first measurement of behaviour. In contrast, the measurements of CSS following deprivation may have been taken, by chance, close to the optimum time for measuring the acute effect of the change (1 to 5 hours after).

4.4.3 Approach test

Boxes reduce the stress a cat shows after an approach test - *CSSfin* reduced by an average of 0.25 (mean days 1,4 – mean days 2,3). *CSSstart* is reduced by 0.08, though this is not significant. It should be noted that the observer did not go further forward than the front of the cage, so whether or not boxes reduce CSS peaks caused by cage cleaning or members of the public trying to stroke the cat is unknown. Nonetheless, boxes appear to reduce the stress caused by an observer attempting to interact, which improves the cats' welfare and may make the cats easier to rehome. Boxes also reduce the number of cats fleeing (class '5') in *apprecoded*, which agrees with the above. This reduction in class '5' is not caused by the cat already being at the back of the cage because it is in the box, since class '5' cats nearly always moved from the pod to the run or *vice versa.* This reduction would seem to show a positive welfare impact for the cats, and increase their chances of being rehomed. Since *friendliness* classes '4' and '5' (all class '5' *apprecoded* had *friendliness* '4' or '5') were not significantly different on box days, this indicates that the box does not make these cats more friendly, but does decrease their perceived need for flight.

Boxes increase the number of cats scoring '3' in *friendliness*, ignoring the observer. It is unclear which categories reduce to create this rise, but cats scoring '3' rarely move towards or away from the observer during the test, so it seems as if cats are more reluctant to leave the box either to withdraw further, or to approach an observer. This may indicate that boxes might make relatively unstressed cats less easily homed, though no effect on *apprecoded* was found.

The author did not actually touch or pet the cats even if they wished contact during the approach test. Since Turner (1991) found that complying with the other partner's wishes was often reciprocated, this lack of response from the author may have reduced the likelihood of the cats approaching in later tests (score 1 did decrease, over the 4 days, though this was NS at $p = .083$).

4.4.4 Scan

Cats at NCC spent more time at the front of the cage than cats in the other shelters. This was probably due to the entire floor surface being covered with Vetbed, as opposed to a section towards the back of the cage as in the other two shelters. Although this flooring is more expensive, both initially and in terms of ongoing cleaning, it does encourage cats to come to the front of the pod, which may make them easier to rehome. Boxes did not make cats more likely to be in the pods as opposed to the runs. There were however two specific accounts of cats in NCC that spent all the daytime in the outside run (even in the winter), but were observed to be inside when the boxes were present, and that reverted to their previous behaviour once they were taken away again. Boxes may therefore be especially useful for such cats for reducing their stress at being in the pod, and possibly aiding their rehoming.

When boxes were present, many cats used them. More cats were in the boxed area on box days (Fig. 4.36), though some were in the area on days 1 and 4 also as it was where the blankets (Bath) and cat beds were (Axehayes). More telling is the *exposed* graph (Fig. 4.35) that shows that nearly half the cats are inside the box when present. This reduces how many cats are out in the open, so may affect homeability.

Only a few cats used the box to perch on. This may be due to the motivation to use the box as an enclosed place to hide being stronger. There was no significant difference between baseline and deprivation days, so cats easily reverted to their previous behaviours.

4.4.5 Cortisol

There was no significant difference between baseline and box samples. Given the small sample size, the test had low power. Nonetheless, a large effect would probably have been discovered if present.

4.4.6 Baseline CSS ("*stress*")

Day 1 CSS is a good predictor of CSS on later days – cats with *high stress* on day 1 tend to have high CSS on days 2-4. The effect of *stress* is additive, so *high stress* cats do not have a greater reduction in stress than *low stress* cats. Cats with high baseline stress were generally less friendly, and were somewhat less likely to approach the observer during the initial phase of the approach test, though neither of these was affected by the presence of box. There were no significant effects of *stress* on *CSSupdown*, *Position*, *Exposed* or cortisol variables. There is no evidence that box provision has a greater effect on cats which were most stressed during the baseline.

4.4.7 Overall

Boxes encourage cats which are particularly unwilling to interact with humans to stay inside their cage when approached by a human, which suggests a welfare benefit to the cat as well as a possible help in rehoming. Most cats do spend time inside the boxes when present. This constrains cats towards the back of the cage rather than the front, which may negatively affect rehoming. There is no obvious way to ameliorate this, as moving the box further forwards would probably encourage cats to hide behind it instead. Few cats were observed using the perching aspect of the box, though this may have in part due to the hiding aspect being more important to the cats, and also its insulating properties – the temperature inside the box was 1-2°C higher than the general pod temperature when the cat was inside, and the box also shielded the cat from draughts caused by air conditioning or otherwise. Also, for NCC cats, the top of the box may have seemed cramped compared to the expanse of blanketed space available to them.

Although there was no measurable effect of box presence on CSS or CC, removing the box did lead to an increase in CSS, which suggests that it was a valued resource for the cats. Based on this, the author recommends that similar boxes are used in shelters. Due to possible rehoming issues (which have not been investigated fully), shelters may wish to restrict box use to admissions (see Chapter 3), or just for particularly stressed cats. Since boxes reduce score '5' in *apprecoded*, cats which are poorly socialised to humans might particularly benefit. The five 'very large' cats in Axehayes were too large for the box, so the author recommends shelters to either make all boxes slightly larger, or provide larger boxes for these cats.

5. Discussion

5.1 The effects of acute stressors

All previous comparable studies have concluded that admission to an animal shelter, or other confinement in a restricted and unfamiliar environment, is acutely stressful for cats (e.g. Kessler and Turner 1997, Rochlitz *et al.* 1998a). The studies reported in Chapters 2 and 3 both support this conclusion. From Chapter 2, cats showed a decrease in both physiological and behavioural signs of stress over the 8 days following admission, with both CC and CSS appearing to plateau around day 6. Over time, the cats became more friendly, purred more, and were more willing to move during an approach test. Within-cats, physiological stress in admissions correlated positively with being at the back of the cage, being alert, having a high CSS, and correlated negatively with friendly behaviours. Comparing between cats, those with high CSS on day 1, the time of most acute stress, were less likely to make a postural movement in the approach test or to purr, but more likely to deliberately ignore the observer in an approach test, which may be a deliberate sign of non-challenge. Cats with the fastest decrease in CSS also had the fastest decrease in *fear*.

Due to physiological plasticity, the cortisol response to a continuing stressor such as admission to a shelter may rise only briefly after initial admission (e.g. Ladewig and Schmidt 1989). It is therefore possible that the decline in CC with time was a result of this adaptation rather than a reduction in the cats' stress, though the simultaneous changes in behavioural variables suggest that this was not the case.

In addition to increasing CSS, stress due to admission caused cats to be at the back of the cage, and inhibited affiliative behaviour and purring. Behavioural inhibition is a common response of cats to shelters (McCune 1992). Although there was no direct correlation with time after admission, CSS also correlated with *fear* and *ignore,* which suggests that the stress caused by admission also caused cats to become more fearful and withdrawn / submissive in social contacts.

Similar studies have been conducted on kennelled dogs. Wells and Hepper (1992) conducted behavioural assessment of dogs over the first 5 days after admission and found that dogs took longer to eat food, were more likely to become agitated and less likely to be relaxed in the presence of an unfamiliar person, on the day of admission compared to days 3 and 5 after admission. No change in activity or alertness over the 5 days was found. Hennessey *et al.* (1997) found that dogs have elevated cortisol levels over the first three days of kennelling compared to days 4-9. These studies indicate that kennelled dogs also suffer behavioural and physiological changes due to the acute stress after admission.

Chapter 3 also revealed a drop in CC over time, confirming that cats are physiologically stressed by admission. CSS decreased, as did *approach*, *firstapp* and *soc1st*, which suggests that acute stress causes cats to become more behaviourally inhibited, and less friendly.

In summary, acute stress caused by admission to a novel, restricted environment in a cattery appears to induce both physiological stress and behavioural signs of stress. Overall, these behaviours, and the high CC shortly after admissions, suggest that acute stress reduces the welfare state of admitted cats.

Additionally, the behaviour of the most stressed cats (as described in Chapter 2) would tend to reduce physical contact with the staff. Since contact should be beneficial for all but poorly socialised individuals, their behaviour may prolong the period for which they are acutely stressed, further diminishing their welfare. Some cats respond differently and may be more active and vocal (McCune 1994), though this may lead to the staff having to control escape attempts and so may not promote beneficial contact with staff. By manual inspection of the data, these active / aggressive cats (Karsh and Turner 1988) were in the minority for this study.

5.2 The effects of chronic stressors

Previous studies which have recorded behaviour and physiology over an extended period of confinement (Kessler and Turner 1997, Rochlitz *et al.* 1998a, Smith *et al.* 1994) have concluded that many cats continue to experience stress for weeks or even months after acute stress would be expected to have diminished due to habituation and behavioural adaptation. All three studies reported in this thesis have included cats that have been confined in shelters for more than one week. However, the environment was not consistent throughout each of these studies; in Chapters 2 and 3 the cats were subjected to a second set of potential acute stressors when they were moved from admissions to the cattery / rehoming area, and in Chapter 4, boxes were provided for all cats and were then removed. None of these studies was therefore ideal for distinguishing between signs of chronic as opposed to acute stress, but some conclusions can be drawn.

No work conducted on dogs indicates when the transfer from acute to chronic stress occurs. Wells *et al.* (2002) showed that changes still occurred after years spent at the shelter, though this study was confounded as dogs showing unfavourable behaviours are less likely to be rehomed. Beerda *et al.* (1999a) showed that dogs which had been individually housed for 2-3 and 5-6 weeks still showed changes in behaviour such as lower postures when challenged and increased circling during a restraint test, compared to baseline measures.

From Chapter 2, transfer to the cattery occurs at different times after admission for different cats, but always at least 8 days after. Studies by Kessler and Turner (1997, 1999a, 1999b) indicate that cats begin to acclimatise by day 5, but have not fully acclimatised by day 14. Similar findings are reported by Rochlitz *et al.* (1997). This suggests that cattery data from Chapter 2, and day 14 data from Chapter 3 will be mostly from the chronic stress period but with a little acute stress. Although Carlstead *et al.* (1993) showed that chronically stressed laboratory cats remained stressed over a 21 day period, the cats were subject to continual acute stressors during this time.

From Chapter 2, transfer to the cattery appeared to have less of an impact on the cats than initial arrival at the shelter, with only CSS significantly increasing on the day of transfer. This lesser effect of transfer may have been due to the cats having become accustomed to shelter life, or accustomed to being moved into a novel shelter cage, though it is also possible that the larger cages in the cattery may have allowed the cats to regulate their stress by using hiding behaviours more effectively. This suggests that measures in the cattery indicate more about a chronic state of stress than the acute stress of admission.

Ignore was positively correlated within-cats with both CC and CSS in the cattery. There are several possible reasons why cats which are chronically stressed are more likely to *ignore*: as a sign of non-challenge; deliberately not looking at the negative stimulus to reduce their negative emotional state; behavioural inhibition due to stress; or they may simply be less interested in interacting. Cats are less likely to be at the back of the cage during approach tests when they have an elevated CSS however – this indicates that high CSS may be due to frustration, with the cats coming to the front of the cage in an attempt to have the observer relieve this by either letting the cat out, or interacting with it. There was no significant correlation with affiliative behaviour however (as there was in admissions), so there may not be a difference once interaction has been initiated by a known human. This difference between admissions and the cattery may distinguish between acute and chronic stress, with high level, acute stress causing cats to be more wary and fearful of their environment and of people, while chronic stress in the cattery, for at least some cats, involves being bored and wanting human contact rather than being fearful of the environment. Furthermore, the observer was well known to the cats by the time they were transferred to the cattery, which may have affected how they viewed him. Brown (1988) notes that a mammalian response to long-term confinement in a small pen is often reduced activity and unresponsiveness. It is not known how long it takes before cats typically respond in this way, and it possible that this boredom, rather than stress, governs why some cats with lower CSS did not approach the observer.

Chapter 4 indicated that cats with high baseline CSS (after at least 15 days in the shelter) were less likely to *approach*, and were less *friendly*. This suggests that chronically stressed cats may be less likely to obtain positive social contact with staff, and may be affected more by caretaking procedures. However, one cannot say from the data which is the causal factor (stress or lack of contact with staff) and which the result – it is also possible that lack of socialisation or a timid personality is the underlying factor for both. Other than showing that cats with high CSS on one day tended to consistently have high CSSs on subsequent days, no other differences were found. This is at odds with the finding from Chapter 2 that high CSS correlated with being at the front of the cage, though that finding was within-cats – it is possible that for most cats which are generally not particularly stressed, their times of most stress may lead them to seek out human contact. The few cats who are still quite stressed may generally be less inclined to seek contact.

So, cats who have high CSS in the chronic stress period are more likely to *ignore* the observer, be less likely to approach and are less likely to be friendly. The decreasing likelihood of cats being at the back of the cage when they have personally high CSS may ameliorate this for cats which are not particularly stressed. These cats may seek human contact to relieve their stress.

5.3 Measures of welfare

5.3.1 Cortisol

Many studies have found that urinary CC rises upon exposure to acute stressors such as unpredictable manipulations on cats (Carlstead *et al.* 1993) and admission to a shelter for dogs (Hennessy *et al.* 1997), as well as chronic stressors such as long term stays (32 days) for cats in a quarantine cattery (Rochlitz *et al.* 1998a) and for dogs 6 weeks after being transferred to individual housing (Beerda *et al.* 1999b). Urinary CC appears to track both acute and chronic stressors well - Carlstead *et al.* (1993) found a continued, elevated urinary CC ratio throughout a 21-day stressful caretaking regimen for laboratory cats, though no studies have looked at whether the HPA axis in cats is down-regulated in the long term (after 3 weeks). Although McCobb *et al.* (2005) found only a very small decrease over time, their analysis may have been overly affected by outliers, and much of the data came from cats which had been admitted for more than ten days, so may have been in the chronic phase of stress at the time of measurement.

Chapters 2 and 3 in this thesis form the first large scale, detailed longitudinal studies of urinary CC in a shelter setting known to the author (work by Rochlitz *et al.* 1998a used 7 animals). Comparisons with previous studies are covered in sections 2.4 and 3.4 respectively. Similar studies have been conducted on dogs: Hennessy *et al.* (1997) found that urinary CC in dogs admitted to shelters fell after days 1-3 and was still falling by day 9. Beerda *et al.* (1999b) found that urinary CC tended to be elevated every week after admission, though this was only significant for week 6 (the last week of the study), implying a cumulative effect of chronic stress. Beerda *et al.* (2000) found that urinary cortisol in dogs housed in kennels was elevated compared to privately owned dogs, even after years of housing, but Gaines *et al.* (2003) found that while CC did increase acutely in dogs taken from homes to military kennels, it had declined to near its original level within 10 days.

CC falls over time following admission to a shelter (Chapters 2 and 3), and may take longer than CSS to 'wind down'. In Chapter 2, cats with high CC were generally more likely to be alert, to ignore the observer, and less likely to purr during the approach test. In admissions only, they were also more likely to be at the back of the cage. This wide range of behaviours shows that CC can be used as a global measure of stress, though it correlates with different parameters to the CSS (see below). It should be remembered that baseline CC is rarely affected by the environment due to physiological plasticity (Carlstead and Sheperdson 2000) so may not be suitable for measuring chronic stress caused by being in a shelter *per se* (though see Beerda *et al.* 2000, above). Chapter 3 showed no effect of treatment group, or of any of the demographic data tested, on CC. Even though box provision had a global effect on stress, this reduction was only in behavioural measures. Although the large between-cat variation in CC may have masked any treatment effects, such effects cannot have been large else they would have been detected. CC decreased over time, which suggests that levels were increased by admission, then decreased over time and were still decreasing after the first week. Chapter 4 showed no effect of provision of boxes on CC, though the very small sample size gave this test low power.

Chapter 3 values were similar to Chapter 2 despite the different assay methods (Chapter 2 was ELISA, Chapter 3 RIA), both being around 7 (molCort:molCreat x 10^6) on day 2 or 3 and decreasing to a plateau of around 5.5 by day 6 or 7. Day 14 values for Chapter 3 declined even more, to a CC of 4.10 by day 14. Chapter 4 means were 3.89 and 4.66 respectively, for baseline and with box, for long-stay cats. Since urine from Chapters 3 and 4 was analysed by the same laboratory and can be directly compared, this suggests that CC ratios for Chapter 3 had reached close to plateau by day 14.

Chapter 3's value of 3.9 (molCort:molCreat x 10^6) for long-stay cats is still higher than the baseline for laboratory cats of 1.1 found by Carlstead *et al.* (1993), though slightly lower than the value of 5.0 for domestic cats in a home environment, and long-stay quarantine cats found by Rochlitz *et al.* (1997). Since laboratory cats have to some extent been bred for tractability, this might have led to Carlstead *et al.*'s cats having generally low CC values.

In conclusion, urinary CC continues to decline in shelter cats until after the first week of admissions. By day 14, urinary CC changes little from day to day and may therefore be assumed to be close to baseline, though this may be due to physiological adaptations rather than a decrease in internal 'stress' (e.g. Ladewig and Schmidt 1989). This fear is allayed as CC also correlates with a range of behavioural measures including CSS. Due to large between-cat variation (e.g. in Chapter 3, day 6/7 had a mean of 5.61, and a variance of 4.69), it may be unsuitable for between-subjects experimental protocols unless large sample sizes are used.

5.3.2 CSS

No analogous scales for measurement of stress for other species are known to the author. Flight distance in farm animals is conceptually similar (e.g. cows, Mulleder *et al.* 2003), since it measures fearfulness towards human approach, which is one aspect of observer-assessed CSS. Other scales such as flightiness and confidence sliding scales used during approach tests on cows (Rennie *et al.* 2003) are also analogous to aspects of the CSS, though no score known to the author assesses the combined effect of complex environmental stressors in a similarly integrative way.

The CSS used in this thesis underwent minor revisions between each experimental Chapter as the author refined it to increase both intra-observer consistency, and apparent consistency within each cat. Direct comparisons of means with Kessler and Turner 1997, 1999a and b are impossible due to shelter differences, though the author's scoring does appear to have less spread then theirs: Chapter 3 scores with a day 1 mean of 3.69 and a day 14 mean of 3.31 show less variation than Kessler and Turner's (1997) scores of around 4.7 on day 1 and around 2.8 by day 14, though again this might be due to differences between shelters, or the range of cats that they accept. If it was due to the author's scoring not showing enough of a spread, this will to some extent be ameliorated by (and explain the need for) the inclusion of half categories in his scoring of CSS. Although McCobb *et al.* (2005) echoed one of the author's reservations with the CSS (difficulty scoring cats resting with eyes closed), they appeared not to have altered the CSS to take these into account, so their subsequent conclusion that the CSS is not a suitable instrument for measuring stress may not apply to all assessors. Ottway and Hawkins (2003) used the CSS to look at cats resident for a month or more at two different shelters, and expressed no dissatisfaction with the measure (other than discarding some data collected at below 15°C). Their scoring of cats had nearly all data at either CSS = 2 or 3, with roughly equal numbers of observations at each. Their means of around 2.4 and 2.6 for single housed and communally housed cats respectively were lower than the author's for Chapter 4, which also addressed long stay cats. In addition to idiosyncratic differences in using the CSS, these lower values may have been due to differences between shelters (shelters studied by Ottway and Hawkins had huts and sleeping boxes where cats could hide very well), and Ottway's practice of spending two weeks at each shelter to habituate cats to his presence before commencing behavioural observations.

Although no other studies (to the author's knowledge) have been published using the CSS, the author feels the CSS could usefully be updated for the sake of future researchers.

The baseline CSS of 3.18 in Chapter 4 is below the day 14 estimated marginal means of 3.26 for CSS and 3.35 for *ScanCSS* in Chapter 3. This indicates cats may not have reached baseline by day 14, although NCC and Axehayes (being less busy shelters at the time of study) would be expected to have lower CSS in any case. It may also in part be due to continued refinement of the CSS as scored by the author between these two studies. The effect of CSS on welfare is open to debate – Ottway and Hawkins consider a two-week stay where cats have a CSS of 3 or below as acceptable. From the author's personal observations, cats frequently attain a CSS of 3 in acutely stressful domestic situations, and more rarely a score of 4. Although suffering only occurs when noxious stimuli are prolonged (Dawkins 1990), a cat which is very tense due to admission to a shelter can almost certainly be said to have poor welfare at that time. For long-stay cats which generally have CSSs of over 3, their quality of life may be poor (at least during the period that the shelter is open to the public).

From Chapter 2, cats with high CSS were less friendly towards the author, and showed more fearful behaviours (in part due to the overlap between CSS and *fearful*). Cats with high CSS were more likely to ignore the author, and less likely move during approach tests, which may be due to behavioural inhibition. In the cattery, they were less likely to be at the back of the cage (interpreted as being more desirous of social contact).

In conclusion, the CSS is a useful behavioural measure that weakly correlates with urinary CC and shows a smooth decline following admission. Given the definitions of the higher stress levels, it is especially useful for measuring levels of fearfulness, though it also correlates with a suite of other behaviours related to soliciting social contact. This link with fearfulness may make timid cats more likely to score high CSSs, though these cats may be those most likely to be stressed by shelter life in any case.

5.4 Boxes

5.4.1 Boxes and acute stressors

Cats frequently show a high motivation to hide following admission to a shelter (McCune 1992), or exposure to other stressors (Carlstead *et al.* 1993). Although cats will use hiding places if provided (Smith *et al.* 1994), no published study has looked at the effect of box provision, and it is unknown how much of an effect boxes will have on stress. The only study known to the author, an M.Sc. thesis by Kry (2003) reported only change in CSS, not actual levels, so the exact effect of box provision is hard to judge. The cats provided with boxes had more of a decrease between day 1 CSS and subsequent days' CSS than the cats not given boxes, the difference being around 1 scale step on the CSS for most of the study. These boxes were similar to those of Chapter 4, and were provided in admissions, which may explain why the effect of box provision was greater than that found in Chapter 3, which used boxes that were more open.

From Chapter 3, cats use the box when present, though they do not use that area of the cage dramatically more than cats without boxes. There was no significant effect on friendliness or approach behaviours (which may depend largely on the individual personality of each cat, making statistically significant results difficult to obtain), and although there was an effect on the staff approach test, the conclusions about the effect of providing a box were unclear. Boxes make cats less stressed as measured by videoed CSS. This effect peaks on the day of admission, then decreases to a more or less constant value over the first week. Boxes reduced day 1 *ScanCSS* also, and cats with boxes had a faster decrease in *ScanCSS*. This agrees with the findings of Kry (2003). This result may be due not only to the boxes limiting the cat's perception of vulnerability, but also their allowing the cats to have some measure of control in how visible / vulnerable they are – this perception of control may be as important as the actual effect. For *ScanCSS*, this illusion of control may have been destroyed when the author touched the cat during social tests, which may explain the lack of further reductions in *ScanCSS* in addition to the different specificities of *ScanCSS* and videotaped *CSS* tests.

Although effects on homeability are not of much concern while in admissions, there may be some acute stress immediately following transfer to homing which may cause cats to use the box in a similar manner. Cats in admissions must be visible to staff for regular checks, so cats hiding in boxes might take up more of the staff's time, and require them to open a pod to look in at a cat, which it may find aversive. Lowering the CSS will tend to make cats more attractive to potential adopters (Vandenbussche 2001), so boxes may increase homeability. Cats are generally less exposed when the box is present, though most cats using the box adopt a position with their body inside the box but their head lying outside, which seems unlikely to have a great effect on how the public regard the cats. Cats with boxes may have hidden more, though this is uncertain. This effect is probably due to boxes making hiding attempts more rewarded for cats, rather than making cats fearful for longer.

Boxes do not have any apparent negative effects on welfare, so are recommended as a way to increase the welfare of shelter cats. They do not appear to make cats more or less likely to approach, nor make them any more or less friendly – it appears that the 'global' reduction in stress becomes replaced by a specific reaction to humans in these circumstances.

5.4.2 Boxes and chronic stressors

As mentioned in section 5.2, the 'chronic' stress of day 14 in Chapter 3, and of Chapter 4, is a mixture of chronic and acute stressors. To the author's knowledge, no other study has looked at the effect of boxes on long stay cats (as in Chapter 4), though Kry (2003) found that the difference between decreases in stress (using day 1 as a baseline) was even more pronounced on day 14 than on days 1-7, with cats provided with boxes having a greater decrease. This was the opposite of the result from Chapter 3 that boxes increase cats' CSS on day 14. One postulated reason for the increase described in Chapter 3 was that the public show more interest in cats in boxes due to their novelty value (if most cats do not have boxes, those which do may attract more attention). Kry does not give data on the proportion of cats in her study shelter with boxes compared to those without boxes on day 14, so this hypothesis cannot be tested. The other hypothesis was that visitors had to peer closely at cats in boxes. The boxes she used almost completely enclosed the cats, so visitors will have had to peer more closely than those in Chapter 2 to see them. Since the cat is also less exposed, it might still feel safer in the box.

This increase of CSS with boxes present was not found in Chapter 4, with boxes (non significantly) lowering CSS. This decline could have been due to most cats in each shelter receiving boxes at the same time, which should greatly reduce any effect of the public showing more attention to cats in boxes, and the increased hiding possibilities offered by the box might have overcome any effect of the public peering closely at the cats.

The average increase in CSS caused by removal was significant (0.163 rise) though still small. The increase may have been a response to the removal of a valued resource (suggesting the box improved welfare even though no CSS decrease was found), or a response to the high number of people visiting the shelter on day 4.

Other effects of adding a box include a reduction in cats fleeing upon approach. Though this may not apply once the pod door is open, and thus have little effect once the public or caretakers enter the cage, it may have a significant welfare impact on the times when the public or caretakers simply look into the cage. It is possible that boxes do not affect the cat's internal levels of fear, or the acute stress caused by a human's approach, but merely inhibit the behavioural response (fleeing) - i.e. a cat may be just as fearful, but perceives the pod as more secure (due to the box), so requires a greater stimulus (entry into the pod by a human) to trigger flight. However, *CSSfin* was lower on box days, which argues against this interpretation - cats were less stressed after an approach when the box was present. This effect may not have occurred in Chapter 3 due to the author touching the cats even if they were in the box. There is no effect on whether the test increases or decreases the cats' stress (*CSSupdown*), which suggests that boxes do not affect how a cat views an approach test (positive or negative event), but do reduce how stressed the cat is by the test, which will improve the cats' welfare. Although increased stress *per se* does not indicate whether the stimulus is positive or negative, *CSSfin* was significantly positively correlated with *apprecoded* and *friendliness* for all days (all $p < .001$, all correlation coefficients over 0.668) which confirms that cats which were more stressed by the test found it more aversive.

There was no increase or reduction on friendliness overall, though cats were less inclined to show their reactions towards the observer, as they remained in the box rather than approaching or withdrawing. This does not suggest a particular effect on welfare. Kry (2003) similarly found no effect of boxes on approach tests or friendliness.

Cats with high baseline stress tend to have higher CSS on subsequent days, but this has no interaction with box provision or removal and is likely to be due to the personality of each cat. Similar results were found with the approach test, with less stressed cats being more likely to approach, less likely to withdraw and more likely to be friendly. This agrees with the Chapter 2 results that high CSS cats are less affiliative. In this case, it is impossible to tell if this is as a result of being more stressed, or a lack of socialisation leading to both higher scores in the approach test and higher CSS. Cats with higher baseline stress are not significantly more likely to use the box, which suggests that some of the stressors (such as lack of contact) do not cause the cat to become fearful, or that box use is largely unconnected with chronic stress.

There was anecdotal evidence that very stressed cats found the box useful. There were two cats in NCC that came indoors during the day only when a box was present (and none only went outdoors), and reverted to their previous behaviours when it was removed. This suggests that for some cats which are poorly socialised to humans the box may help to make them feel more secure in the pod. These cats will be more stressed by admission to the shelter (Kessler and Turner 1999a). This may be especially beneficial during the winter.

In terms of affecting homeability, the box only reduces CSS slightly, if at all - the drop from baseline to days 2 and 3 was small (.052 of a CSS scale point), so even if there is an effect of box on CSS, it may not be worth the extra time and money required, though the benefits may be great for a small number of cats, as the box does reduce the number of cats fleeing at the start of the approach test. This may increase their homeability, as it increases their visibility to the public. Boxes increase score '3' in *friendliness*, apparently at the expense of scores 2 and 4. While this may be considered unhelpful for cats which are generally friendly, the box may allow cats which are not well disposed towards humans to appear less unfriendly. Cats were less exposed on box days, with a decrease in being fully exposed but an increase in *exposed* category '3'; in the box with head visible. Although the cat's face is visible, the cat is definitely harder to see than when the box is not present, which may well make the cat less easy to rehome. *Position* confirmed that cats spent less time at the front of the cage, and more time at the back where the box is, both inside and on top of the box. It is possible that box use was artificially high due to novelty value (e.g. De Monte and Le Pape 1997), though this cannot be tested with the data from either this study, or that of Chapter 3 (time series data are confounded with the drop in acute stress). Relatively few cats used the shelf aspect of the box, preferring to be inside it. The decrease in visibility for cats, together with the evidence that boxes have most effect in the approach test for cats which are nervous of human contact, suggests that boxes may provide a net benefit for these cats, but may slightly impair rehoming for cats which are not nervous.

5.5 Social contact

The effects of social contact of cats in shelters is largely unstudied – Carlstead *et al.* (1992) subjected 8 cats to 8 days of 'stress', consisting of relocation, physical restraint, and jugular venupuncture. Half of the cats experienced a drop in urinary cortisol which was interpreted as "[these cats] may have been responding to the rewarding properties of being held during blood sampling". These four cats were the most affiliative with humans out of the eight. No other studies on the domestic cat known to the author have looked at the effects of providing social contact. Kessler and Turner (1999a) found that cats which are poorly socialised to humans tend to be more stressed during the first week after admission (no later days were studied) than those which are well socialised.

Mertens and Turner (1988) studied the social interactions of 240 unfamiliar test humans on nineteen laboratory cats and found significant differences between the way male and female humans behaved in the social setting, though there was no effect of the human's sex on how the cats responded to them. The sex of the cats' caretakers was not reported however. Hennessey *et al.* (1997) found an effect of gender – dogs petted by a female had a decrease in plasma cortisol over the 20 mins, while those petted by a male had an increase, which led to no overall effect on cortisol levels on the population of dogs. Two different petters of each gender were used, so the effect may have been one towards specific humans rather than to specific sexes. Hennessy *et al.* hypothesised that this difference could have been due to different odours of male and female humans, that possibly petting behaviour of female humans is more soothing than that of males, or that it was due to the females having experience in training dogs with behaviour problems. In Chapter 3, although the observer was male, and all the approach test volunteers were female, the cats had both female and male caretakers at the shelter, so would have been familiar with both sexes during their stay. Any previous biases would still have been present however.

Chapter 3 investigated the effects of repeated social contact with cats in rescue shelters. This was only investigated in cats exposed to acute stressors, and reduced ScanCSS over the first fortnight as measured by the human who gave them social contact. No effect was found on reactions to other humans, although no effect towards the author in equivalent tests was found either. This suggests that the minimal contact that half the cats received, on top of scans and other observations, may have been enough for the cats to regard the observer as a desired partner for contact, and that 20 minutes of handling each day did not increase this, except as measured by ScanCSS. Hennessy *et al.* (1997) found no overall effect of 20 minutes of social interaction with an unfamiliar human on plasma cortisol levels in dogs.

Since the effect continued to day 14, where the treated cats had not had their 20 minutes of social contact for at least the previous 5 days, this strongly suggests that the effect is due to cats becoming more familiar with the observer, rather than a global effect of reducing their stress. Similar work and has been done on other species: in pigs, Pedersen *et al.* (1998) found that 3 minutes of stroking and patting upon approach to a handler increased approaches to the handler, though did not affect approach to an experimenter unknown to the pigs in an approach test, compared to a minimal handling group. The average day time concentration of free plasma cortisol was lower in the petted group than the minimal handling group.

Although shelter staff generally spend some time in each cat's pod for caretaking duties, the cleaning procedure is aversive to many cats. Staff may become associated with this and other unpleasant stimuli such as being taken to the vet, as well as pleasant stimuli such as feeding. Chapter 3 shows that there are definite welfare benefits to the cats of social contact. It is unknown whether social contact from individuals other than the caretakers will have this effect.

5.6 Demographic variables

Although none of the Chapters explicitly aimed to investigate demographic factors, they were included as factors in GLMs and ordinal regressions for chapters 3 and 4, so some results can be discussed.

5.6.1 Strays

In Chapter 3, strays were less likely to approach both unknown staff and the observer. They also appear to have been more generally stressed on day 14 (higher videoed CSS). Since this CSS was taken at the end of the day when the public had been present, this may be a reaction to close observation by many unknown humans. This is backed up by Chapter 4 results which show that strays were more affected by the box, showing a more pronounced lowering of CSS on box days (Fig. 4.23). All of these confirm that strays appear to be stressed by humans, both in approach tests, and *en masse* in the shape of the public. This could be due either to strays being less used to human contact due to their time as a stray, a consequence of that part of their personality that had encouraged them to become strays in the first place, or a mix of both. There was no significant difference caused by being in a shelter *per se* (days 1 and 4). McCobb *et al.* (2005) found no difference in Urinary CC ratios between stray and owned cats, though since most samples were taken after the first week where most acute stress occurs, physiological adaptation may have masked any differences which were initially present.

Wells and Hepper (1992) found that the public considered stray dogs less desirable, and although Vandenbussche (2001) did not investigate the effect of cats being strays, she did find a trend for cats which approached an unknown observer to be more likely to be rehomed, though this was not significant (22 cats out of 28 that approached were rehomed, compared with 37 out of 61 that did not approach). So, strays are often less fit for shelter life and may be harder to rehome. Cats which are harder to rehome are sometimes put into the part of the shelter where most of the public go, or have notices put up about them ('Cat of the week', or similar), to encourage a higher proportion of the public to view the cat. For cats such as strays which may be poorly socialised to people, such efforts may be somewhat counter-productive as the extra human contact may make them more stressed, and so less likely to be rehomed. Staff encouraging only a few particularly suitable / sympathetic individuals to look at the cat may be productive.

5.6.2 Age of cat

From Chapter 3 data, older cats are less likely to approach unknown staff and the observer, and have generally higher CSSs while in admissions. These do not carry over into rehoming (though the rehoming tests had less power due to a lower N), nor were any effects found in Chapter 4. This suggests that older cats may simply adapt to shelter life more slowly, such that they reach plateau later. McCune (1994) found the opposite, that old cats tended to have lower Cat-Assessment-Scores (the progenitor of the CSS) during the first two days in the shelter. Kessler and Turner (1997) found no effect of age on cats' adjustment to the boarding cattery. Comparing their results with McCune's, they postulated that the difference might be due to differences between the studies in average ages or space allowances, or in scoring CAS / CSS: "The differentiation between a restful and a stressful sleeping posture may have lead to a different scoring of young and old animals", p. 253. Similar reasons might explain the difference between McCune's results and those of Chapter 3.

Older cats were more likely to be more friendly by day 14 (in the rehoming area of the cattery, see Chapter 3), despite being less likely to approach (or more likely to approach in the second phase of the approach test), though this was not tested in Chapter 4. Since most *firstapp* scores were between '1' and '3', older cats may be less likely to approach not because they are more likely to be in categories '4' and '5' but because they are generally less active, and thus less likely to approach even if they desire social contact.

Vandenbussche (2001) only investigated age in categories of 'kitten' (less than 6 months old), 'adolescent' (less than 2 years) and 'adult' (over 2 years), so her result of kittens and adolescents being more likely to be rehomed does not usefully apply to this study. Shelters sometimes anecdotally report that older cats are harder to rehome, though it is unclear which particular attributes (or perceived attributes) are causing this. Possible vet bills, a likely bereavement for children to cope with and wanting a more active cat were among those cited to the author by the public.

5.6.3 Sex of cat

Females were more likely to approach the observer on day 3 after admission (Chapter 3), and showed the same trend on day 7 (though this was NS), though the opposite effect on day 14 was found. Females were also more friendly, though only on day 14. There were no effects on CC, general stress, or ScanCSS, so the reason for these differences and the change in them is unclear. The p-values for sex were never particularly low, so may have been due to chance alone. Females had lower CSS in Chapter 4, though this became NS at $p > 0.1$ if the significant sex*stray term was removed. The effect of sex is therefore largely unclear, though there is no evidence for female cats being more susceptible to acute stress in the way that female dogs are (Beerda *et al.* 1999b).This may be due to most cats in the study having been neutered by their owners when young.

5.6.4 Neuter / entire status

There was only one discovered effect of being entire, with entire cats being part of the unexplained drop in CSS around day 5 (Chapter 3). Since this drop had no between-cats effect and the reason for it is unclear, the effect of neuter status is also considered to be due to chance.

5.6.5 General

So, all other things being equal, old or stray cats are less likely to approach both known and unknown humans in admissions, so may require more careful handling shortly after admissions.

5.7 Effects on rehoming

Nearly all shelters studied had waiting lists of cats needing space in admissions, with waiting times sometimes lasting for weeks. The reasons for admissions may vary, but in many cases the prolonged wait may be a welfare problem for the cat and / or the owner. Any measures that make shelter cats more attractive to potential adopters will help shelters rehome cats currently there and free up space for new cats. Euthanasia is sometimes applied to shelter cats as a more or less routine procedure after a cat has spent a certain amount of time in the shelter, or if it remains fearful. This is more common in countries where the throughput of cats is more rapid, such as North America and Canada. Although euthanasia is not a welfare problem for the cat *per se*, shelters would prefer not to have to apply it, and it may be perceived as a welfare problem for the cats' previous owners, and affect donations by members of the public.

Vandenbussche (2001) found that cats with high CSS were less likely to be rehomed than cats with low CSS. Any procedure that reduces the stress of cats in shelters in the rehoming area will therefore tend to increase their homability. Shelters vary in the minimum time cats spend in admissions before being allowed into the homing area, though this is very rarely less than three days (pers. obs.). Some acute stress will occur shortly after moving and may be a significant proportion of time spent in rehoming before euthanasia, though in UK shelters which rarely euthanase, it is the reaction to chronic stressors that determines how long the cat stays at the shelter for. For long-stay cats (Chapter 4), cats which are more stressed are less likely to approach humans, and more likely to withdraw during an approach test, though it is unclear if this was lack of socialisation causing the high CSS, or *vice versa*.

Boxes can be used to reduce cats' acute stress response to initial admission. This effect is greatest on the day of admission, though continues until at least day 7. Boxes can therefore be used to help reduce the stress of initial admission, which should reduce the CSS and make the cats easier to rehome. No effect of Box was found on likelihood to approach or friendliness, although Box cats were more likely to have a decrease in *approach* between days 3 and 7 than Nbox. The increase of CSS on day

14 (Chapter 3) is a possible concern – no similar effect was found in Chapter 4, and may be removed by having more enclosed boxes (q.v. Section 5.4.2).

Although no effect of box addition on CSS was found in Chapter 4, boxes reduced the number of cats fleeing upon approach, and reduced the stress immediately after approach (*CSSfin*). Cats being ambivalent during approach tests was increased, which might reduce the chances of generally friendly cats being rehomed, but might increase them for generally unfriendly cats. The estimate of friendliness during approach tests did not include the observer attempting to touch the cats, so any effect of actual contact with unfamiliar humans is unknown, though the lack of effect in Chapter 3 suggests that it is likely to be small.

The anecdotal evidence that the box encouraged two very stressed cats to use the pod during the day suggests that box provision for stressed cats may help them feel secure enough to be inside. At most shelters known to the author, cats which are inside are more likely to be seen by the public, especially during winter when the outside runs are cold. Vandenbussche (2001) noted a non significant trend for cats to be rehomed more easily when inside rather than out, though it is unclear what is the causal factor in this case.

Cats with boxes are generally less exposed and less likely to be at the front of the cage, though this effect may decrease with time as the novelty of the boxes lessens. Work on dogs (Wells and Hepper 1992) suggests that this may make animals less attractive to the public, and Vandenbussche (2001) also found a non-significant trend for cats which were at the front of the cage during approach tests to be rehomed, compared to those at the back. This suggests that providing unstressed, socialised cats with boxes might make them less attractive to adopters, but that the gains in welfare noted above might outweigh this for more stressed cats. These cats will include those which are poorly socialised to humans (Kessler and Turner 1999a) and/or have timid personalities. Since adopters frequently mention the character of the cat as a reason for their choice, letting the cats show themselves 'in their best light' is important. Boxes are a tool shelter staff can use to help some cats appear less scared of potential adopters.

Social contact in admissions gave no significant increase in friendliness or likelihood to approach, so does not appear to aid rehoming, although this was not tested in the homing area on long-stay cats. Social contact may be useful to relieve boredom for such cats.

5.8 Future work

5.8.1 Box design

The exact mechanism by which boxes reduce stress is not known. Cats which are stressed appear to prefer hiding places which are: elevated; large enough to fit the entire body into, though generally quite small (tactile stimuli from all sides); darkened (this may be a side effect of being small, of having a 'roof' or a desirable property in its own right); and that block line of sight (although cats frequently remain able to orientate themselves so they can look out). To what extent the 'ideal hiding place' in a shelter setting is determined by the cat's previous experience of hiding, and how much is instinctual, is unknown. Whether the cat evaluates properties such as blocking line of sight and whether this is judged by the size of the occluding object, being able to see the unpleasant stimulus, or some other method, is also unknown.

Stressed cats with no boxes (Chapter 3) frequently tried to hide behind the back edge of the blanket, or in the corridor, neither of which effectively blocked line of sight to the front of the pod, but would have given the cat tactile stimuli (pers. obs). Some cats tried to burrow their heads into the far corner of the pod / corridor, facing away from the pod front, others positioned their heads so they could see out through the front door of the pod.

Each of the properties above (elevation, size, darkened and blocking line of sight) can be investigated separately. For example, hiding places that staff can see into easily for making checks would be useful – making boxes (or the front wall of each box) out of clear plastic tinted dark or medium red (cats' eyes do not contain red cones, so it is assumed they perceive red things as darker than other colours, Loop *et al.* 1987). The entire pod could be darkened by the addition of tinted plastic (lighting gel or similar) to pod windows, though since cat eyes adapt to uniformly dark conditions very well, they may not perceive the pod as being particularly dark.

Blocking line of sight could be investigated by using clear vs opaque walls. As mentioned above, it is not known whether the walls of a box need to be fully opaque for this effect, or whether a semi-opaque wall would work as well. Whether cats like to be able to look through the window at the front of the pod without having to expose themselves (e.g. through a small hole at the front of the box, or through a semi-opaque box) is of interest, as is how they judge when they are sufficiently 'hidden'.

Although the position of the box in the cage is not a design feature *per se*, placing a box towards the front of a cage may encourage cats to be closer to the public (so long as they do not hide behind the box).

5.8.2 Social contact

Social contact in shelters is generally either from staff, or from 'cat cuddlers', human volunteers who visit semi-regularly to stroke cats, generally in homing areas only. The effects of such contact on general stress, and on reactions to approach by unknown humans would be of interest.

5.8.3 Personality

Although the possible effects of different personalities have been discussed, they were not explicitly tested in any of the experimental Chapters. How the personality of each cat (active / timid / confident) affects its response to social contact with known and unknown humans, and the effect of box provision is of interest – sociable, confident cats may gain little benefit from boxes but may enjoy social contact with known and unknown humans, while timid cats may be helped by the constant presence of a box but may need a more careful introduction to social contact.

5.9 Recommendations to shelters

5.9.1 Box use and design

Boxes can be used to reduce cats' acute stress response to being admitted to a shelter. Their greatest effect is on the day of admission, but continues until at least day 7. This suggests that shelters can use boxes to help cats acclimatise to shelter life. Many shelters only give cats boxes if they are still very stressed by day 3 or 4, which is too late for most cats to gain the maximum benefit of the box.

The boxes used in Chapter 3 were designed to allow the cat to be seen easily, so as not to affect rehoming, though if used in admissions, visibility of the cat is of less concern, so more enclosed boxes, similar to Chapter 4 boxes, could be used, and may have a larger effect – Kry (2003) reported a difference of 1 scale point in CSS between cats enriched with boxes similar to Chapter 4 boxes and those without, during the first week after admission. Based on a few cats at Axehayes being very cramped in the box, the author would recommend building slightly larger boxes, or making two sizes of box. The boxes were designed to be good for hiding, but for most cats who are not particularly stressed, larger boxes which allow performance of the more relaxed postures might be preferred. The platform aspect of the boxes seemed to be poorly used, though this may have been due to it being warmer inside the boxes, or the platform seeming cramped compared to the floor space available. Cats which fled to the outside run when the author approached them were often hindered by having to go through the side hole in order to reach the cat flap, and one cat appeared to switch preferences from being inside the box to being on top of it after a particularly harrowing attempt to flee during an approach test which involved upturning the box.

The boxes used for Chapter 4 would make it difficult for staff to thoroughly check over a cat that stays in its box without moving the box, though this could to some extent be remedied by enlarging the front hole.

Although boxes have a definite benefit for cats shortly after admissions, the effects for long stay cats are unclear. Stress in the long term will not be due to the novel nature of shelter life, but because aspects of it remain stressful. These might be the presence of unfamiliar humans (or of all humans) during the day, the presence of other cats, or the enclosed, routine nature of every day. The last factor is particular to long stay cats and is not one that boxes are likely to help, though the first two frequently also cause acute stress to most cats soon after admission, and it seems credible that box use might help these cats suffering from the first two, in giving the cat somewhere it can 'escape' to. Some factors of box use such as being less exposed might reduce a cat's homeability, although the benefits of box provision may outweigh this, especially for cats that remain stressed. Many shelters provide chronically stressed cats with 'igloos' in which they can hide, though these generally restrict the public's view of the cat more than Chapter 3 boxes. The higher CSS of Box cats on day 14 of Chapter 3 indicates that some care when placing boxes in rehoming should be taken, though no such effect was found in Chapter 4.

Since staff often have to remove the box entirely to take the cat for veterinary checks, this may reduce the cat's perception of the box as a secure area, so cats in the box should be disturbed as little as possible.

5.9.2 Social contact

Social contact reduces CSS when measured by the human who gave the social contact. This shows a clear welfare benefit to staff spending time with the cats, though the individuality of each cat must be taken into account, as some may not be well socialised to humans and can easily find contact aversive. Whether this will deliver a benefit related to cats' reactions to humans who have not given them social contact is unknown.

6. Summary

Experiment 1:

1. Both CC and CSS fell over the first week after admission. They were positively correlated within cats but negatively correlated at the population level. This may indicate different coping strategies.

Experiment 2:

2. Boxes reduced CSS as recorded by video camera, with the greatest effect occurring on the day of admission and continuing until at least day 7. They also lowered CSS on the day of admission as measured in the presence of observer, and produced a faster decrease in CSS over subsequent days.

3. Increased social contact with the observer (20 minutes daily versus 1 minute) reduced CSS, but only when measured by the observer, and not when measured remotely by video.

4. Social contact had no effect on an approach test by an unfamiliar person, though cats with boxes became more likely to approach and less likely to withdraw over the week than those without. When tested by the observer, there was no effect on the approach test, or friendliness during the first minute of contact.

Experiment 3:

5. The CSS of long-stay cats was affected by the presence of a box, with CSS increasing when the boxes were removed, though this effect was small.

6. Boxes made long-stay cats less likely to make either an approach or withdrawal during approach tests. This may be beneficial for cats which are timid and likely to flee when approached.

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Appendix 1

Using partial correlations to remove relationships between intercepts and slopes that result solely from interdependence

From Chapter 2, p. 76, Table 2.16: "in the data, a variable's intercept and slope are frequently correlated, probably due to floor and ceiling effects. This correlation means that intercept and slope are not independent and may lead to problems with interpreting data. Partial correlations were used to remove relationships that resulted solely from interdependence".

As an example, the correlations between CC and CSS in admissions will be considered. Reformatting Table 2.16a gives Table App.1:

Table App.1 Spearman correlation coefficients for curve intercepts (predicted day 1 values) and slopes. Equation $y=b0+b1.\ln(day)$, intercept = b0, slope = b1. Admissions data only, days 1-8, n=23.
** Correlation is significant at the .01 level (2-tailed).
* Correlation is significant at the .05 level (2-tailed).
Significant +ve correlations shaded in purple, -ve correlations in yellow.

	CCINT	CCSLO	CSSIN	CSSSLO
CCINT	*	-.846**	-.327	.376
CCSLO	-.846**	*	.458*	-.477*
CSSINT	-.327	.458*	*	-.787**
CSSSLO	.376	-.477*	-.787**	*

CC *int* and CC *slo* are negatively correlated (coeff -0.846, $p < 0.01$), presumably because cats with a higher CC upon admission can drop further than those with low CC on day 1. CSS *int* and CSS *slo* are also negatively correlated, probably for the same reason (coeff –0.787, $p < 0.01$). Comparing CC and CSS, there is a negative correlation between CC *slo* and CSS *slo* (coeff –0.477, $p < 0.05$). CC *slo* and CSS *int* are also positively correlated (coeff 0.458, $p < 0.05$), but since each variable's slope and intercept are also correlated, it is unclear which of the relationships is causal.

Since the correlations between each variable and its slope are expected and have obvious causal links, it can be assumed that these are correct. So we either have: a causal link between CSS *int* and CC *slo*, which creates an artefactual correlation between CC *slo* and CSS *slo* due to the correlations between CSS *int* and CSS *slo*, and between CC *int* and CC *slo*; or a causal link between CC *slo* and CSS *slo* which creates an artefactual correlation between CC *slo* and CSS *int* due to the correlation between CSS *int* and CSS *slo*; or a mixture of both. To separate out these factors, partial correlations were carried out as shown in Table App.2

Table App.2 Partial correlations for data in Table App.1. Coefficients and 2-tailed p-values shown

(CC *slo* and CSS *slo* from table 13a):	Coeff = -.477, p = 0.021
CC *slo* and CSS *slo* controlling for CC *int*:	Coeff = -.319, p = 0.147
CC *slo* and CSS *slo* controlling for CSS *int*:	Coeff = -.336, p = 0.127
CC *slo* and CSS *slo* controlling for CC *int* and CSS *int*:	Coeff = -.172, p = 0.458
(CC *slo* and CSS *int* from table 13a):	Coeff = .458, p = 0.028
CC *slo* and CSS *int* controlling for CC *int*:	Coeff = .275, p = 0.216
CC *slo* and CSS *int* controlling for CSS *slo*:	Coeff = -.049, p = 0.828
(CC *int* and CSS *int* from table 13a):	Coeff = -.327, p = 0.128
CC *int* and CSS *int* controlling for CC *slo* and CSS *slo*:	Coeff = -.071, p = 0.761

From inspection, the correlation between CC *slo* and CSS *int* becomes highly non-significant and even reverses sign when CSS *slo* is controlled for. In contrast, the correlation coefficient and p-value for the correlation between CC *slo* and CSS *slo* is relatively unaffected by controlling for either CC *int* or CSS *int*. The conclusion is that there is a true causal relationship between CC *slo* and CSS *slo* (Figure 2.9). The correlation between CC *slo* and CSS *int* is entirely due to the correlation between CSS *int* and CSS *slo*. There is also a non-significant negative correlation between CC *int* and CSS *int* (Figure 2.10).